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TITLE: **DEVELOPMENT OF A COMPREHENSIVE THERMOREGULATORY MODEL FOR EXERCISE AND RESTING RESPONSE IN WOMEN EXPOSED TO THERMAL STRESS WEARING MILITARY CLOTHING**

PRINCIPAL INVESTIGATOR: Richard R. Gonzalez, Ph.D.

ASSOCIATE INVESTIGATORS: Laurie A. Blanchard, William F Allison, J.A. Gonzalez, Thomas Endrusick, Robert Wallace, and Avraham Shitzer, Ph.D.

CONTRACTING ORGANIZATION: U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760-5007

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Richard R. Gonzalez, Ph.D.

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Natick, MA 01760-5007

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13. ABSTRACT *(Maximum 200 words)*
Three separate studies were conducted in this project: a resting study on six women; several thermoregulatory modeling projects and an exercise/cold stress study on nine women. Effects of the menstrual cycle on heat loss and heat production (M), core (Tc) , and skin temperature responses were studied in six unacclimatized, women nonsmokers (ages 18-29 yr), resting supine. Each women was exposed to a cold ramp (Ta= 20°C to -5°C, -0.32 °C/min, rh 50 ±2%,V=1m/s) in the follicular phase (F=days 2-6) and mid luteal phase (L= days 19-23) of her menstrual cycle. It was shown that extensive peripheral vasoconstriction in F during early periods of the ramp generally elevated resting Tes above thermoneutral levels. Shivering thermogenesis was highly correlated with Mean weighted skin temperature), and finger temperature. There was a reduced slope in the M as a function of mean body themal drive in L experiments when dressed in BDUs ($P<0.02$) and BDU+BDOs ($P<0.01$). Heat flux was higher and cold debt was less in the L phases when dressed in BDUs. The modeling study showed that, several cold model equations appear to be applicable for depicting shivering thermogenesis provided the dominant thermal drive is not from decreasing core temperature and cold adrenergic drives and individuals are within a range of body fat between 8% to 30%. In the exercise study, nine healthy women were exposed to a cold challenge as in the resting experiments during their early follicular (F) and mid luteal (ML) phases. The women walked on a treadmill at a work rate of 32 % VO2max dressed in BDUs + parka. During submaximal exercise/cold stress, control of ventilation and cardiac output was found to be linked with altered core temperature (Tc) but the sweating and heat exchange requirements are driven by thermoregulatory adjustments appearing during a woman's menstrual cycle.

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FOREWORD

As documented in the Institute of Medicine, Recommendations for Research on the Health of Military Women 1995 report, a paucity of information is present on the heat-adverse and cold-adverse effects or hypothermia in women. Additionally, it has been estimated that " women represent about 14% of the total active duty forces of the United States military services and 16% of the reservists, totalling approximately 340,000 people", (Defense Women's Health Research Program Home Page, 1998).

The ability to predict cooling rates and shivering thresholds and judge trade-offs in tasks *vis à vis* climate at various work rates and military clothing systems (e.g., BDU, BDO, and the Extended Cold Weather Clothing Systems) and derive appropriate stay times is a critical need. It is also poorly documented whether environmental risk is driven higher in military women because clothing systems and personal protective gear (such as gloves, boots), meant for universal use do not meet special thermoregulatory requirements for women which can be compromised by the menstrual cycle, peripheral and central circulatory differences, and other needs. Many of these clothing systems were secured from data garnered primarily in males-based research. The need for physiological and operational information in military women is especially required as missions of most service personnel become supplemented in the future towards a more global rendering of humanitarian or non-combat aid. It is crucial that adequate thermoregulatory models become available predicting both male and female response (taking into account both genders' intrinsic differences), covering wide cold and heat stress environments, and which are practical in forecasting or deriving preventive medical risks.

The information characterizing female responses to cold stress derived from research conducted in this report should help in the building of an adequate mechanistic thermoregulatory model that is useful over wide climatic zones and military field situations or applicable to various programs involved in integration of soldier simulation thrusts directly pertinent to servicewomen. The payoff to this research is also vested in the database that was secured that helps close the gap to answering gender differences required in modeling human performance.

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Richard R Gaydos
11/21/98

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INTRODUCTION

The reproductive system has a clear and important role in altering thermoregulation in women, particularly when internal body temperature becomes elevated during the luteal phase compared to the follicular phase of the menstrual cycle. In the luteal phase, thermoregulatory responses of women are characterized by alterations in core temperature (T_c) thresholds affecting the onset of specific physiological effector responses during exercise, heat exposure and cold exposure (Hessemer,1985;Kolka,M.A. et al., 1989;Stephenson, 1985). Elevated core temperature thresholds controlling the onset of sweating and skin blood responses are consistent with a higher internal body temperature reference point evident in the luteal phase, which may compromise thermoregulation during prolonged exercise or warm exposures in the luteal phase (Kolka, 1989;Pivarnik, et al., 1992; Stephenson, 1985). Several studies (Hessemer, 1985;Kolka and Stephenson, 1987; Kolka et al., 1989; Stephenson, 1985) carried out in warm conditions during exercise show that when T_c and skin temperatures are elevated, heat loss mechanisms become activated and there occurs increased sudomotor drive and increased blood flow to the skin surface in the mid- to late luteal phase of some eumenorrheic women.

Human thermoregulatory responses during cold stress are generally determined by the person's ability to maintain thermal balance (Bittel and Henane, 1975;Gagge and Gonzalez, 1996; Hammel, 1968; Stolwijk and Hardy, 1977; Vallerand et al., 1992). Since homeotherms control internal body temperature by energy exchange, energetics plays a key role in response to cold. Often depicted by a curve showing metabolic heat production as a function of steady-state air temperature (Hammel, 1968), this pattern gives little

indication of controlling mechanisms governing thermoregulatory responses or interplay of other systems with the internal core temperature. Little data exist quantifying female responses to cold stress. During cold stress, shivering by gross muscular contraction may or may not be sufficient to maintain T_c. T_c and skin temperatures interact in a unique fashion either as constant temperature multipliers or in a summative fashion to increase metabolism (Boulant and Gonzalez, 1977;Hammel, 1968). However, other than limited studies (Bartelink et al., 1990; Hessemer and Bruck, 1985; Stephenson and Kolka, 1985), there is a scarcity of information on how reproductive hormones during various stages of a woman's menstrual cycle influence thermoregulatory responses to cold. This lack is especially evident characterizing whether women during specific stages of the menstrual cycle display a differential thermosensitivity to a cold challenge. This thermosensitivity may be described by a change in slope to a decreasing internal or skin temperature affecting a given heat loss or heat production response. The integrated mean body ($\bar{T}_{b,i}$) or core thermosensitivity of a thermoreguatory response is defined in this project as the amount of change in the specific dependent response for each unit change in $\bar{T}_{b,i}$ during cooling. The change in slopes of the respective dependent response curves above a given reference $\bar{T}_{b,i}$ describe the various thermosensitivities. The shivering response is generally determined by a plot of excess shivering ($\triangle M$) vs either skin or T_c (Benzinger et al., 1963;Boulant, 1996; Hammel, 1968; Hessemer and Bruck, 1985). Primarily looked at in this part of the project was the peripheral thermosensitivity of shivering thermogenesis.

The thermoregulatory control system has primary inputs from central (preoptic/anterior hypothalamic) and peripheral (e.g. skin) receptors. The regulated variable, T_c , is generally recognized as the defended variable (Benzinger et al.,

1963;Hammel, 1968). Extensive research (Boulant,1996; Hammel, 1968) demonstrates that most afferent thermal signals coming from the skin and deep body core, following a short time lag, are sensed and integrated by the preoptic/anterior hypothalamus. Changes occurring in hypothalamic neurons activating effector responses such as cutaneous vasoconstriction, shivering, and other forms of heat conservation (often behaviorally driven) become stimulated as well (Hammel, 1968). Skin temperature is set by the extent of blood flow to a site, thermal conductivity, and environmental temperature and will reach a level imposed by the ambient conditions (Gagge and Gonzalez, 1996; Hammel, 1968; Stolwijk and Hardy, 1977). Various studies (Benzinger, 1963; Boulant, 1996; Hammel, 1968) have revealed that there is an interaction of hypothalamic temperature with skin and deep-body temperatures in the initiation and control of various thermoregulatory responses. Skin cooling has been shown to alter the hypothalamic thermosensitivity driving increases in metabolic heat production and cutaneous vasoconstriction (Hessemer and Bruck, 1985; Stolwijk and Hardy, 1977; Tikuisis et al., 1991). Fluctuations of estradiol and progesterone levels, and changes in their relative ratios, during a woman's menstrual cycle also participate in mediating cutaneous vascular responses during cold challenges (Bartelink, 1990; Hassan et al., 1987; Hessemer and Bruck, 1985).

When T_c is offset to a higher temperature level in a woman's mid luteal phase, there may exist competitive inhibition in the processing of thermal information in the thermoregulatory system (Boulant, 1996). This competition of thermal afferent information from central and peripheral receptors may blunt the shivering response to cold stress at a given mean skin temperature (Hessemer and Bruck, 1985). Additionally, there is strong evidence that hypothalamic neurons show changes in their local hypothalamic

thermosensitivity during displacements in cutaneous temperature that closely parallel whole body thermoregulatory responses (Boulant and Gonzalez, 1977). That is, the highest slope (e.g. "gain") in a shivering response (Benzinger et al., 1963; Boulant, 1996) occurs when cold T_c is combined with cold skin temperatures and their combined rate of change is rapid.

In this study focus, clothing insulation,(e.g., thermal resistance added to a fixed tissue resistance), is considered as an extrinsic factor which only functions as part of the passive means in the regulation of energy exchange between the body and the environment (Gagge and Gonzalez,1996). If a woman in the follicular phase has a given body resistance (tissue+clothing layer, r_{s1}) and this is increased to r_{s2} by addition of another finite layer of clothing, then the critical temperature is extended and effects of T_c changes affecting shivering thermogenesis are generally diminished. Extension of the lower thermal limits can be done by the addition of more clothing which adds an additional thermal resistance to the skin layer for a limited period of time and severity of cold stress.

Little data exist characterizing female physiological responses to cold stress with exercise. During light to moderate exercise in the cold, performance was found to actually improve and lactate production decreased in the luteal phase (Jurkowski et al.,1981). Also lacking is knowledge about the mechanisms active in cardiorespiratory variables during exercise which are presently difficult to model. Many of the responses may involve changes due to prostaglandins and/or CNS responses to cytokines or other neurochemical substances that would modulate such thermoregulatory responses during a women's luteal

phase particularly during exercise (Hoffman-Goetz and Pedersen, 1994;Cannon and Dinarello, 1985; Foster et al., 1986).

The intial resting experiments were designed to perturb the thermoregulatory system by employing a repeatable cold stress (Gonzalez, et al., 1981). The technique allows quantification of dynamic responses and provides information characterizing cold reactions in women. The resting study had three major objectives: 1) to quantify effective heat exchange responses at two stages of the menstrual cycle in women clothed in two different clothing systems. The hypothesis here is that an increasing level of thermal resistance affects heat loss but does not alter thermoregulatory mechanisms within each menstrual cycle phase; 2) to determine if reproductive hormones, functioning at two stages of a woman's menstrual cycle, affect heat loss during cold transients. The hypothesis here is that heat loss would not become altered between the two stages (Bittel and Henane, 1975; Frascarolo et al., 1990);and 3) to investigate the magnitude of shivering thermogenesis during cold transients. The hypothesis being that peak level of estradiol and progesterone in the luteal phase have significant effects on the T_c reference point, but not the slope of the shivering thermogenesis much like responses in warm ambients (Kolka et al., 1989; Stephenson and Kolka, 1985).

The use of transient responses to ambient temperature (dynamic ambient temperature changes) may reveal mechanisms alluding to why such a response might occur (Gonzalez et al., 1981). Generally, in response to exercise or warm ambients, core and skin temperatures are elevated and heat loss mechanisms become activated with increased sweat secretion and increased blood flow to the skin surface. But in the luteal phase, effect of transient or acute cold stress on the thermoregulatory system often works

counter to the latter responses. Women with a higher internal body reference temperature in the luteal phase may find themselves attempting to eliminate heat by evaporative heat loss or by sensible heat loss. Yet in response to cold stress, skin thermoreceptors are potentiated to increase afferent signals to the hypothalamus calling for initiation of shivering and heat conservation. The greater peripheral body 'shell' can be used as a heat sink or heat source when faced with a warm or cold transient but these properties have never been accounted for in clothing models nor have the peripheral vascular consequences.

The design of the exercise-cold stress experiments (coupling a thermal transient analysis) along with a clothing system (Battle Dress Uniform+ Parka) used by all US Army personnel will allow crucial information characterizing cold reactions in women at two stages of the menstrual cycle. We initially proposed that the thermal sensitivity in the response of shivering thermogenesis as a function of core and skin temperature would become blunted by exercise but not significantly altered by effect of the menstrual phase. Alternatively, we proposed that the rate of sweating as a function of integrated mean body temperature should become elevated during exercise in the luteal phase but depressed as cold skin receptors become stimulated and inhibit sweat gland response. All the data should provide a suitable framework for development and testing of a prediction model of key physiological responses in woman that would improve thermoregulatory, central, and peripheral cardiovascular responses.

A current USARIEM heat strain prediction model has given fruitful forecasting of endurance times, water requirements, and work-rest cycles to a variety of tasks. However, the data are based exclusively on male responses to heat stress (>68 °F). Presently

needed is the development of algorithms and validity studies on females testing the model's equations describing: a) effect of load carriage at low work intensities; b) position of load carried (e.g. coefficients for back, hand, litter carriage, etc.); c) effect of terrain coefficients, grade, and walking speed.

The specific scope of this proposal was to study comprehensive effects of rest and moderate exercise activity and one clothing system in females. New algorithms were developed or perfected for a wider range of temperatures (e.g. cold) which should permit more thorough applications useful for predicting female responses to thermal stress and clothing systems.

Technical Objectives: a) To develop a thermoregulatory simulation system characterizing female responses to cold stress that incorporates effects of peripheral response, clothing and body temperature changes;

b) To address by model predictions how variation in stage of the menstrual cycle modifies thermoregulatory and cardiorespiratory responses to cold stress during rest and exercise that may or may not adversely increase risk of cold injury in women in the services;

c) To establish by model prediction for rest and exercise activities which mechanisms of thermoregulatory control are specifically challenged because of displacements of core and skin temperature, differential skin blood flow response, heat flux, and rate of heat debt during the menstrual cycle in women.

BODY

Study A: Effects of menstrual cycle in resting women dressed in protective clothing ensembles

EXPERIMENTAL METHOD

In the first experimental study six, highly motivated volunteers were recruited from the military test subject pool. Each woman volunteered to participate in the study after being informed of the risks and purpose of the study and after giving their written, informed consent. The protocol was approval by this Institute's Human Use Review Committee (HURC). Women selected completed a questionnaire which incorporated various signs and symptoms related to their menstrual cycle. A complete medical examination was done on each woman prior to any experimentation. Each woman had no history of cardiovascular or respiratory disease, nor complications from irregular menstruation. Before any testing began, each subject had a blood scan to ensure they were not anemic, and a pregnancy test, which was verified as negative.

Each woman completed all experiments. The mean (±SD) age was 21.2 (± 3.9) years, height was 1.65 (± 0.10) m, weight was 60.9 (± 7.9) kg, body surface area 1.66 (± 0.12) m^2, and % body fat was 23.9% (± 2.5 %). Experiments on each volunteer subject were done in the late fall, winter, and early spring between 0700-0900h to control for circadian variation in temperature regulation. The women were normal early risers. Each of the six female volunteers displayed a normal menstrual cycle as defined by regular periodicity (~28-30-day cycles) kept in a daily log book and no subject was taking oral contraceptives. To verify that ovulation had taken place in a given month, each subject recorded her daily basal body temperature upon awakening for the month and continued

throughout completion of the experimental study. Oral temperature was measured twice while resting at the same time each morning (mouth completely shut) using a calibrated, fast responding, automated oral thermometer. Data from an entire menstrual cycle were collected and graphed prior to the study to determine whether oral temperature increased after ovulation (Kleitman and Ramsaroop, 1948). Although basal body temperature monitoring is not a wholly sufficient method to predict ovulation time, higher basal internal temperature is closely correlated with the higher plasma progesterone concentration evident in the luteal phase of the menstrual cycle (Cargille, et al., 1969) and directly applicable to resting values.

Testing in the luteal phase occurred only on days when the resting core temperature was elevated (approximate days 19-23). Testing in the early follicular phase occurred on days 2-6 (day 1 = first day of menstrual flow). The calendar dates initially picked to correspond with a given stage of the menstrual cycle of the woman were verified by post hoc blood analyses of estradiol and progesterone levels. Hormonal data, basal body temperature records, and subject logs were all matched for proper cycle phase at the end of the total study protocol. Experimental data authenticated with the respective hormonal data from each woman were used in the statistical analyses. Hormonal data were culled according to appropriate phase and test day to determine mean differences in precision of measurement by analysis of variance (e.g., inter-assay variablilty) for the two separate test samples from each women. Per cent differences between test days are shown in Table 1 along with the mean concentration levels of the reproductive hormones at each cycle phase.

Clothing ensembles

The women dressed in each of two clothing ensembles devised to add two constant fixed resistances to the womens' skin surface layer. Ensemble A consisted of the U.S. Army issue physical training shorts and underwear plus the U.S. Army T-shirt worn underneath a Temperate Battle Dress Uniform (TBDU). Ensemble B consisted of a U.S. Army Battle Dress Overgarment (BDO) worn over Ensemble A. The clothing insulation values were evaluated separately at three different wind speeds to establish effective clothing heat and water vapor transfer coefficients employed in partitional calorimetric analysis. These heat transfer coefficients were estimated using a regional copper manikin resting supine on a wooden-framed cot supported by parachute nylon webbing which simulated the conditions of the human experiments. The total thermal resistance (R_T) of Ensemble A measured at chamber wind speed of 1 $m \cdot s^{-1}$ (paralleling the human experiments) was 0.21 $m^2 \cdot K \cdot W^{-1}$ (1.33 clo). The clothing resistance of Ensemble A is equivalent to normal civilian trousers and shirt clothing. The R_T of ensemble B, also tested at wind speed of 1 $m \cdot s^{-1}$, was 0.4 $m^2 \cdot K \cdot W^{-1}$ (2.58 clo).

During all experiments, subjects wore a standard U.S. Army Light Duty Work Glove with a five-finger woolen insert which added a constant thermal resistance to the hands. The glove was separately evaluated on the USARIEM copper hand model and had a R_T of 0.13 $m^2 \cdot K \cdot W^{-1}$ (0.86 clo). During all experiments, a standard issue U.S. Army Black Boot and U.S. Army Cushion-Sole Socks were worn on the feet with each clothing system. The thermal insulation of the boot with a sock was analyzed (1.8 clo;R_T = 0.28 $m^2 \cdot K \cdot W^{-1}$) using a regionally heated copper foot in our laboratory. During all experiments, subjects were not allowed to open or ventilate their garments by opening closures, zippers, etc. or

by excessive movement, thereby serving to control against disparate changes in skin surface temperatures and heat flow.

Prior to the beginning of the study, volunteers were thoroughly familiarized with all experimental techniques and had their standing height measured. During these training sessions, body weights were measured so that on the four test days (twice in Ensemble A and twice in Ensemble B), pre-experiment body weights could be easily traced back within 1% of the mean body weight measured during preliminary testing to control for possible effects of hypo-hydration.

Experimental Testing:

Upon arriving at the laboratory each morning, the subject rested on a chair for 30min and a 10-ml blood sample was taken by venipuncture for the measurement of serum 17β-estradiol (E2) and progesterone (P4) concentrations (by radioimunoassay, Coat-a-Count, Diagnostic Products Corp., Los Angeles) to accurately define menstrual cycle phases of each woman (Table 1). Blood samples were quickly processed when taken and frozen. Samples from all women were analyzed in the same batch assay (in duplicate) in order to obviate potential inter-assay variablility as pointed out before. After a blood draw, the previously trained volunteer inserted a polyethylene-encased thermocouple through the nostril channeled through the pharynx into the esophagus to a depth about 25% of her height (in centimeters). Exact placement of the thermocouple at the mid-heart level was verified by following a real-time thermal recording on a computer screen as she slightly inserted and retracted the thermocouple into the esophagus past the initial "hot" spot demarcating a correct heart level point (Stephenson and Kolka, 1985). The women were ask to avoid swallowing by spitting saliva into a cup during the experiments in order to

obviate spurious recordings. Any inadvertent swallows, shown by immediate dips on the computer monitor scan, were later smoothed in the data file by using an exponential smoothing of T_{es} to predict a value based on the forecast for the immediate prior 15s period.

Surface thermistors with calibrated heat flow disks (Concept Engineering, Inc., Old Saybrook, CT) were placed at six skin surface sites (mid-chest, mid-thigh, lateral calf, upper hand, upper arm, and mid-forearm) and area weighted to estimate mean skin temperature ($\bar{T}_{sk}$, Nishi and Gagge, 1970). Separate 28 gauge copper-constantan thermocouples were also placed in the middle finger nail bed and big toe nail bed. The calibrated heat flow disk surrounding each embedded thermistor element determined heat flux from each of the skin sites. Weighted heat flux ($W \cdot m^{-2}$) was calculated from each respective skin site area weighting (Nishi and Gagge, 1970).

Environmental temperatures, esophageal and skin temperatures, and heat flow data were recorded every 15s using a personal computer. Wind speed in the chamber was controlled at 1 $m \cdot s^{-1}$.

Oxygen uptake ($\dot{V}O_2$, $\ell \cdot min^{-1}$) was measured by collection of all expired gases into a 2-min Douglas bag, sampled every 20th min of the transient and twice at 20 °C, once prior to the decrease of ambient temperature and once after some 10-15 min period at -10°C. Heat production ($W \cdot m^{-2}$) was calculated from the expired respiratory parameters obtained, the calorific equivalent of one liter of oxygen, and the DuBois surface area equation (Gagge and Gonzalez, 1996). Subjects were exposed to the lowest air temperature level (-10°C) for some additional 10-15 min. Experiments ceased if a woman

voluntarily withdrew or she was withdrawn because fingertip skin temperature reached ≤5 °C or if esophageal temperature plummeted towards 35 °C. These lower limits were advisory guidelines set by our USARIEM's Human Use Review Committee (HURC) for removal of a given subject from the test for that day.

Heart rates were obtained and recorded every five min from the electrocardiogram measured continuously using chest electrodes (CM 5 placement) interfaced to a telemetry system (Hewlett-Packard 78510A&B).

Specific restrictions placed on the subjects were that they refrain from active exercise, food, caffeine, and medication (including aspirin or any analgesic-antipyretic compounds) consumption ≈10h prior to the experimental testing. If the subject had unintentionally taken any medication, an experiment was rescheduled for the next appropriate calendar day. Body weight and composition were determined by repeated nude weighings and skin fold measurements (Stephenson and Kolka, 1985).

Steady- State and Transient Exposure

All subjects rested supine on a specially designed wooden cot for 15 min of baseline data at 20°C air temperature. After complete instrumentation, an additional resting period began lasting ~20-30 mins until equilibration occurred by having the woman's skin and core temperature remain constant within ± 0.1°C for 10-15 min. After the initial equilibration period, each subject was exposed to the thermal transient by decreasing the environmental chamber ambient temperature ($T_a = \bar{T}_r = T_o$ operative temperature, Ref 12) in a controlled downward ramp (for the 24 runs: $T_o = 17.5 - 0.316 \cdot (\text{min}) + 1.088\text{e-}3 \cdot (\text{min})^2$; $r^2 = 0.98$, SEE ±1.2 °C). Operative temperature (T_o) is the critical variable describing the ambient

environment when clothing is worn (Gagge and Gonzalez, 1996). Dew point temperature was allowed to fall passively during the room temperature decreases. The cooling phase at the lowest target T_o typically continued for 80-120 min (the latter time point when dressed in Ensemble B) at a constant decreased exponential ramping rate of -0.32 °C•min^{-1}. A final exposure time of 10-20 mins at T_o of -5° to -10°C was completed before ending the experiments by a subject's request or in conformity with our Institute's HURC recommendations. All data were truncated to an 80min time point to facilitate statistical analyses.

Heat exchange variables

Partitional calorimetric analyses (Gagge and Gonzalez, 1996; Vallerand et al., 1992) were conducted each 20-minutes of an experiment taking into account each avenue of heat exchange from the heat balance equation (e.g., respiratory and convective heat loss responses combining all respective clothing and heat transfer coefficients) in which:

$$\pm S = M - E_{sk} - (R \pm C) - K, \qquad [W \cdot m^{-2}] \quad (1)$$

where: $\pm S$ = rate of body heat storage (in this study -S, heat debt); M = metabollic heat production calculated from each 20 min $\dot{V}O_2$ interval; E_{sk} = evaporation or insensible (wet) heat exchange which is set by the clothing moisture properties (i_m/clo) (Gagge and Gonzalez, 1996) and evaporative heat transfer coefficients determined from parallel copper manikin evaluations of the garments, the skin to ambient temperature gradient ($\bar{T}_{sk} - T_o$), and by the change in body weight loss and respiratory heat loss; R = radiation; C = convection; K = conduction. R and C combine as sensible (dry) heat exchange which was determined by the

environment, thermal conductance, insulative properties of the ensembles and their respective heat transfer coefficients.

Details of the analysis to ascertain integrated mean body temperature from partitional calorimetry are addressed in the APPENDIX section.

Statistical Methods

Data are reported as means ±SD. For the baseline equilibration periods, mean values of esophageal and skin temperatures, core and skin temperature changes from neutral (ΔTes,$\Delta\bar{T}_{sk}$, and ΔT_{fing}), and M were analyzed by univariate analysis of variance techniques with repeated measures (ANOVA). Whenever a significant F-ratio was found, Tukey's critical difference was employed for post hoc analysis (α=0.05) (SAS, 1990).

Mean data of T_{es},$\bar{T}_{sk}$, heat content (kJ), and heat flux as a function of time during the thermal transient were analyzed by two-way (Ensemble x menstrual phase), (time x menstrual phase), or by three-way (time x Ensemble x menstrual phase) analysis of variance for repeated measures. When the ANOVA indicated significant main or interactive effects, Tukey's Studentized Range, Honestly Significant Difference (HSD) was used to compare means and locate minimum significant differences (α=0.05) between factors and among repeated measurements (SAS,1990).

Regression methods. Regression analyses of M vs $\bar{T}_{sk}$ and T_{fing} were determined using a linear regression for repeated measures taking into account between-subjects differences. Dummy variables were used to encode the different subjects and all the data

were pooled together to estimate a single regression equation (SAS, 1990).

A two parameter, piecewise linear regression analysis was used to fit the data from each subject's shivering thermogenesis (ΔM= M-Mo) as a function of integrated mean body temperature. Each regression coefficient (slope) and threshold point was examined by ANOVA for repeated measures and Tukey's post hoc test as above (Brownlee, 1965).

Parameter model estimation. Maximum likelihood parameter estimation (MLE) was used to determine the respective control coefficients (parameters) derived by independent effects of core and skin temperatures likely to be considered influencing shivering thermogenesis (ΔM=*L*). This technique was chosen as an ideal method of describing ΔM=*L* based on the estimates of three principal control variables: T_{es},$\bar{T}_{sk}$, and T_{fing} (Hammel, 1968; Stolwijk and Hardy, 1977) driving the shivering response throughout the cold transient. It was assumed that the product of a parameter value times a given variable {ΔT_{es}, ΔT_{sk}, and ΔT_{fing}) in the data set are all independent terms that equally weight the final likelihood function (ΔM=*L*). Also assumed was an initial model statement that ΔM is based on proportional control (Hammel, 1968) either by summed linear effects or multiplicative effects from the three variables. In the MLE analysis, starting values of control parameters (P_1,P_2,P_3) and their respective constants (ΔTsk1 , ΔTes2, and ΔTfing3) are first set to default values based on a given model statement. Each subsequent iteration seeks to find the minimum sum of squares residual (SSR) by a Quasi-Newton derivative procedure. This is done by differentiating with respect to a given parameter and equating each derivative to zero

(e.g., $\partial \log L/\partial P_1 = \partial \log L/\partial P_2 = ... \partial \log L/\partial P_n = 0$). Iterations terminate when the values of the parameter estimates in the iteration procedure fail to change. This yields the maximum

likelihood estimator of all the parameters. The method also generates a R^2 = (1-Residual SSR/totalSSR) of each model equation.

MLE is a useful method used to optimize unknown parameters in a probability model where the response variables are dichotomous and the predicted variable is a probability (Brownlee, 1965).

RESULTS

Skin temperatures and esophageal temperature responses. Extensive peripheral vasoconstriction and rate of fall in $\dot{T}_{sk}/\Delta t$ of some -0.1°C/min occurred during all thermal transient runs. During the initial thermoneutral equilibration periods, $\bar{T}_{sk}$ was not significantly different between phases for a given ensemble. Final $\bar{T}_{sk}$ observed in both phases with Ensemble A (23.2±0.6°C) was lower when compared to Ensemble B experiments (27.4±0.3°C) ($P<0.05$).

In Ensemble A experiments, a higher T_{es} was evident in the luteal phase compared to the follicular phase (36.9±0.05°C > 36.6±0.16°C, $P<0.01$) during the basal resting period (at -30min). During the initial phases of the transient, the drops in $\bar{T}_{sk}$ were associated with elevations in T_{es}. Figure 1 shows that ΔT_{es} initially rose in the early time periods of the transients with Ensemble A. ΔT_{es} was elevated higher at 40,70, and 80 min of the transient in the follicular phase compared to the luteal phase ($P<0.05$).

In experiments with Ensemble B, ΔT_{es} was higher ($P <0.05$) during the follicular phase compared to the luteal phase at the 70th and 80th min of the transient. Towards the final time points (min 70-80) ΔT_{es} began to decline markedly with the cold stress.

Metabolic Heat Production. Steady-state metabolic heat production (M,$W \cdot m^{-2}$) in

the women was not significantly different during equilibration time periods (-30min) when wearing either ensemble A or B in either follicular and luteal phases. Mean values were:ensemble A-follicular: 47.16 $W \cdot m^{-2} \pm 5.11$ SD; ensemble A-luteal : 47.28 $W \cdot m^{-2} \pm 4.69$ SD; ensembleB- follicular : 44.2 $W \cdot m^{-2} \pm 5.13$ SD;ensemble B BDO-luteal, 45.9 $W \cdot m^{-2} \pm 6.2$ SD). These values ranged from 5-13% which are not significantly different from the basal heat production values ($M=41.85\ W \cdot m^{-2} \cdot °C^{-1}$) reported for 25 y old females (Hessemer and Bruck, 1985).

Figs. 2 and 3 show that total heat production (M, $W \cdot m^{-2}$) was closely correlated ($P<0.0001$) with the reduced ($\bar{T}_{sk}$) and (T_{fing}) in all of the cold transient experiments. Shivering thermogenesis (ΔM) (where, $\Delta M = M - M_{basal}$, $W \cdot m^{-2}$) was determined from each women's response to the cold ramp and plotted as a function of ($\bar{T}_{b,i}$) as described in the APPENDIX section to derive regression coefficients (6). Table 2 shows the mean results.

The slope of shivering thermogenesis response to integrated mean body temperature ($\Delta M/\Delta \bar{T}_{b,i}$) in the luteal phase of the menstrual cycle in each woman was attenuated during experiments with Ensemble A ($P < 0.02$) and with Ensemble B ($P < 0.01$). The integrated mean body temperature threshold ($\bar{T}_{bi,\ o}$) was not significantly different between phases or ensemble worn.

Heat Flux

Figure 4 shows the weighted heat flux data plotted at 20min intervals from all the experiments. During the first minutes of the cold ramp with Ensemble A in the luteal phase of the women, the heat flow through the garment occurred at an equivalent time point (12-13 mins) as in the follicular phase of the women. Into the 20th min ($T_o \approx 12 \pm 1°C$) and throughout the cold transient, heat flux was displaced toward a higher level in the luteal phase compared with mean values observed in the follicular phase ($P<0.05$). During experiments with Ensemble B, heat flux values were increased by 60-70% ($P<0.05$) above basal values, but mean heat flux at each time point of the transient were not

significantly different between phases.

3 way ANOVA (time x phase x Ensemble) followed by Tukey's HSD indicated that mean weighted heat flow values were higher at each time point and cycle phase with Ensemble A ($P < 0.05$).

Rate of Heat Debt

Figure 5 shows the results of whole body rate of heat debt (-S,kilojoules, kJ) calculated for each 20min period of the transient. Heat debt was determined by partitional calorimetric analyses, accounting for each woman's M, body weight, %body fat and surface area, T_{es}, and $\bar{T}_{sk}$ as explained in the APPENDIX section.

ANOVA revealed significant differences in rate of heat debt from basal time periods. The women in the follicular phase with ensemble A exhibited higher rate of heat debt at 40,60 and final time points ($P<0.05$) in comparison with similar time points in the luteal phase. In experiments with Ensemble B, rate of heat debt was greater than values at basal time points ($P<0.05$), but there were no phase differences throughout the transient.

Prediction equations for shivering thermogenesis (ΔM, $W \cdot m^{-2}$).

The variables: T_{es}, ΔT_{es}, $\bar{T}_{sk}$, and T_{fing} were shown to be independant factors influencing the shivering response (Figs 1-3). For this reason, an attempt was made to develop a unifying algorithm to describe ΔM as a function of the respective skin and core temperature thresholds and control constants. Since the shivering thermogenesis was highest in the experiments with Ensemble A, this data set was looked at for distinct differences possibly related to cycle phase. All data were analyzed by an iteration procedure employing maximum likelihood parameter estimation as explained in METHODS. An acceptable criterion for a model equation based on summative or

multiplicative construct of the independent variables was a derived $R^2 \geq 0.9$ from the lowest sum of squares residual (SSR) (Brownlee, 1965).

Threshold values initiating excess heat production due to shivering thermogenesis (ΔM) were generated from the data set. In the follicular phase: thresholds $T_{fing,0}$ =26.5±0.3°C and $\bar{T}_{sk,0}$ = 32.5±0.2°C were found. In the luteal phase: thresholds were$T_{fing,0}$ =21.5±0.3°C with $\bar{T}_{sk,0}$ = 31±0.9°C . These values were found to be offset to lower temperatures ($P<0.002$). $T_{es,o}$ thresholds were not significantly different between phases and mean values = 36.9 ±0.1°C.

Prediction equations established by MLE are:

Follicular Phase (day1-6), $\Delta M_{fol} \geq 0$:

$$\Delta M_{fol} = [P1F \bullet (\bar{T}_{sk} - 32.5) + P2F \bullet (T_{es} - 36.9)] \bullet P3F \bullet (T_{fing} - 26.5) \quad W \bullet m^{-2} \quad (2)$$

{P1F=0.35; P2f=-0.85;P3F=0.9}

R^2 = 0.91, Sum of Squares Residual (SSR)= 1,776

Luteal Phase (day 19-25), $\Delta M_{lut} \geq 0$:

$$\Delta M_{lut} = [P1L \bullet (\bar{T}_{sk} - 31.0) + P2L \bullet (T_{es} - 36.9)] \bullet P3L \bullet (T_{fing} - 21.5) \quad W \bullet m^{-2} \quad (3)$$

{P1=0.65; P2=-2.28;P3=0.59}

R^2= 0.86, SSR=2,200

The analyses indicate that T_{es}:$\bar{T}_{sk}$ thermal sensitivity (ratio of P2F>P1F) governing the shivering responses was 2.42 $W \bullet m^{-2} \bullet °C^{-1}$ in the follicular phase and 3.5 $W \bullet m^{-2} \bullet °C^{-1}$ in the luteal phase (↑~44.6 %) . That is, sensitivity to a lowering of core temperature is still a stronger factor in the control of the shivering response than T_{sk} alone (Fig 1). T_{fing} was shown to be a significant peripheral multiplier to the summed

effects of core and skin thermal inputs affecting ΔM in both phases. P3F > P3L in the follicular phase was higher compared to the luteal phase (by some 53%).

DISCUSSION

The general results of the resting study suggest that a woman's metabolic response to a lowered skin temperature and lowered integrated mean body temperature is significantly attentuated during the mid luteal phase of her menstrual cycle when endogenous levels of estradiol and progesterone become elevated. It was found that effect of clothing, that does not impede water vapor transfer and is equivalent to the thermal resistance of street clothing (e.g., worn by 80-90% of the population), does not alter the physiologic mechanism underlying this attentuation. The study also confirmed the fact that addition of further layers of clothing (simulating additional body fat) influences shivering thermogenesis and rate of body heat loss primarily by passive increase of thermal insulation (Tikuisis et al., 1991; Vallerand et al., 1992). The general effect of the added clothing insulation is that at a given lowered skin, less heat production is required to maintain deep body temperature and rate of heat debt is diminished as a function of time of the cold stress. In the mid luteal phase of a woman's menstrual cycle, elevations in endogenous levels of estradiol and progesterone may promote specific alterations that potentially influence the thermoregulatory center controlling heat loss and heat production.

A higher esophageal temperature evident in the luteal phase of the women during the basal resting period (at -30min) prior to exposure to the cold transient was expected from the many studies shown previously in unclothed women during various warm and cold exposures (Hessemer and Bruck, 1985; Kolka and Stephenson, 1997; Kolka and

Stephenson, 1985). The interesting finding from the present study was the pattern in which core and skin temperatures modulate the thermoregulatory mechanisms during initiation and progression of the cold transient in the two menstrual cycle phases in the women. This study showed that there were several critical heat exchange mechanisms modified during the luteal phase. Several of these are worth discussing in depth: peripheral vs central thermoreceptor influence on the shivering themogenesis, rate of heat flux and heat conservation mechanisms, rate of heat debt, and thermal mechanisms affected by possible central nervous system mediation or influenced by specific hormonal activity.

Clothing resistance and shivering responses. We hypothesized that intrinsic thermoregulatory mechanisms activated by the hormonal changes appearing during the two menstrual cycle phases would probably not become modified during cold stress by addition of clothing. Adding a finite thermal resistance to the skin layer by clothing blunts somewhat the heat exchange properties but not the consequences of thermoregulatory effector responses (shivering, vasoconstrictor activity). During the two stages of the menstrual cycle, the primary effects of the cold transient persisted in stimulating skin and internal body thermoreceptors. Changes in heat production and heat loss responses still remained controlled by alterations in core and skin body temperatures during the two stages of the menstrual cycle.

In general, maximum vasoconstriction of the peripheral blood vessels increases thermal resistance of the body's total skin layer by a value ~1.3mm thick, which is equivalent to wearing a light sweater over the whole body (Gagge and Gonzalez, 1996). Based on assessments using a copper manikin, the added insulation by Ensemble A to the

skin layer upon maximum vasoconstriction by the women would likely result in a combined resistance (fat+tissue+clothing) of about 9.3mm and some 17mm with Ensemble B. The ANOVA followed by post hoc Tukey HSD analyses showed that the higher thermal resistance of Ensemble B significantly decreased sensible heat loss from the skin surface to the ambient. Additionally, the added clothing resistance diminished heat debt by adjusting the lower critical temperature (e.g., ambient temperature where net metabolic heat production rises) necessary for shivering thermogenesis.

The hypothesis that clothing resistance would not modify thermoregulatory responses was confirmed by the heat flux and rate of heat debt changes (Figs 4 and 5).

Shivering responses. Since the esophageal temperature of the women in each phase was elevated in the cold transients, more so in the follicular phase with Ensemble A, it would appear that the stimulus for increased heat production was in large extent peripheral in origin. Maximum likelihood parameter estimation confirmed that a strong peripheral drive was active in controlling shivering thermogenesis during supine rest in the women. The analyses suggest that as long as the esophageal temperature remains near thermoneutral levels (or ΔT_{es} from basal is ~ ±0.15°C), the summed effect of shivering thermogenesis is highly correlated with a combined effect of both $\bar{T}_{sk}$ (weighted without fingers and toes) and effect from cold acral sites (finger temperature). Skin temperatures from acral sites appear to have an thermal effect on ΔM which is more prominent than their surface area. Decreasing finger temperature below a threshold temperature reference point also has a strong multiplicative influence on overall thermal drive affecting the total shivering response (eqs 2 and 3). As evident in figs 2 and 3, absolute heat production (M, $W \cdot m^{-2}$) was strongly

correlated with the decreases in mean skin temperature and finger temperature during the cold transient.

The reciprocal response of a core temperature rise to skin cooling has been frequently displayed in small animals and humans in which the heat capacitance is limited due to small surface area to volume ratio (Benzinger, 1963; Gagge and Gonzalez, 1996; Hessemer and Bruck, 1985; Vallerand et al., 1992). Hessemer and Bruck also found a reciprocal effect of core temperature to skin temperature decreases in women exposed to cold temperature step changes. In our study, the response was most clearly apparent during the follicular phase experiments when the women were clothed in Ensemble A having the lower thermal resistance (Fig 1). During experiments in the luteal phase with Ensemble A, ΔT_{es} increases due to peripheral cooling were less ($P<0.05$) than that apparent during experiments in the follicular phase at the 40-80th min of the transient. The strong peripheral influence by finger and $\bar{T}_{sk}$ on total metabolic rate was still evident, but dampened, during experiments with Ensemble B in both the follicular and luteal phases (Fig 2 and 3). The blunted response may be due to an outcome of the overall higher skin temperature level by the increased thermal resistance, amounting to twice that of Ensemble A. The consequence of increased extra thermal resistance to the skin surface only served to impede skin heat transfer to the ambient but did not substantially influence the mechanisms governing efferent responses by the thermoregulatory system. These data suggest that integration of the central and peripheral thermal drive was influenced more by the hormonal changes occurring during the two stages than by the clothing resistance added to the skin surface.

Shivering thermogenesis vs integrated mean body temperature. This study also revealed

important information regarding the effectiveness of a change in core temperature relative to a change in mean skin temperature in producing an elevated shivering response during the two stages of the menstrual cycle. In slightly clothed women exposed to a step change of room temperature from 32°C to 12°C, Hessemer and Brück (1985) found that the shivering response was strongly altered by both core and skin temperatures and there occurred a shift in the threshold for shivering to a higher level in the luteal phase. They attributed the shift in threshold for the initiation of shivering to an increase in basal metabolic heat production. They revealed that a decreased EMG response to cold stress also occurs in the luteal phase. Unresolved from their study was whether the slope of the shivering response to skin or core temperature (e.g. the peripheral thermosensitivity or core thermosensitivity of the thermal response) becomes modified as a consequence of the hormonal changes present during the luteal phase. In their study, mean body temperature (termed "Tb(es)") was calculated by a classic 1:4 weighting of skin and core temperature (Burton, 1935), which is questionable for use in cold air studies on women and only valid for steady-state conditions as addressed in the APPENDIX section. This present study confirmed Hessemer and Brück's (1985) results of shivering response being tightly correlated with skin temperature. However, our results indicate that in the luteal phase, decreases in the shivering thermogenesis occur as a function of decreasing integrated mean body temperature ($\bar{T}_{b,i}$) determined from summed heat balance. Unlike Hessemer and Brück (1985), our results found that during the luteal phase, basal metabolic heat production was unaltered and there was no statistically significant shift in the $\bar{T}_{b,i}$ reference point controlling the initiation of shivering. The diminished $\Delta M/\Delta \bar{T}_{b,i}$, and lack of any $\bar{T}_{b,i}$ reference temperature shift, clearly suggests a re-adjustment of overall heat balance

balance possibly brought about by any thermogenic effect by peak hormonal levels of estradiol and progesterone during this menstrual cycle phase.

Several studies have pointed out that hypothalamic warming reduces or even suppresses shivering in a cold environment (Boulant, 1996; Boulant and Gonzalez, 1977; Hammel, 1968). Boulant and Gonzalez (1977) showed in rabbits that the shivering response, modified by a warming and cooling of the preoptic hypothalamic area, was affected by the skin and core temperature. Preoptic heating decreased the hypothalamic thermosensitivity inducing the shivering thermogenesis when colonic and skin temperatures were warm. Preoptic cooling, however, increased the hypothalmic thermosensitivity when both core and skin were cold. The present study showed that shivering response to a given $\bar{T}_{b,i}$ signal is reduced in the luteal phase when the warming activity on the hypothalamus would be most likely prevalent. In response to cold skin calling for increased metabolic heat production, the higher weighted influence of deep body thermoreceptors, possibly acting within a warmed hypothalamic area via thermogenic action of enhanced endogenous estradiol/progesterone levels in the mid luteal phase, could have a dominant role in inhibition of the shivering response.

It could be that the peak shivering response indicated by high peripheral thermal sensitivity occurs primarily at low core and skin temperatures operating during the follicular phase when both levels of estradiol and progesterone are low (Fig 2 and 3).

Role of skin and internal temperatures in the shivering response. Our study showed that in the luteal phase compared to the follicular phase the shivering response to a lowered integrated mean body temperature becomes attentuated. Extent of absolute heat

production was also affected by the increased thermal insulation (Figure 2) and thus some of the noise in the attentuation of shivering response may be attributed to the fact that less heat production is required to maintain $\bar{T}_{b,i}$. The interesting fact from the parameter estimation analyses of data during both menstrual cycle phases is that there appears to be a separate contribution from skin, esophageal, and finger temperatures (representing vasoconstriction in acral sites) influencing the shivering response.

Shivering responses has been found to be predicted by linear dependency between $\bar{T}_{sk}$ and tympanic, spinal, rectal, or esophageal temperatures (Benzinger, 1963; Hammel, 1968; Stolwijk and Hardy, 1977; Tikuisis et al., 1991). Benzinger (1963) first showed in resting men that the shivering thermogenesis was an inverse function of tympanic temperature (T_{ty}) so that no shivering appeared if $T_{ty} > 37.2°C$. The higher the tympanic signal >37.2°C, the less was the effect of a low steady-state skin temperature from a reference level of 33°C. In other schemes, thermoregulatory control functions are generally represented by quasi-lumped parameter influences from skin and core thermoreceptors (Stolwijk and Hardy, 1977; Wissler, 1985). In such schemes, the output of the controller (shivering thermogenesis) is proportional to the difference between the sensed value (skin and core thermal signals), the controller variable (e.g., T_{hy}, hypothalamic temperature), and various thermal reference or "set" points (Hammel, 1968). Hammel (1968) proposed that shivering thermogenesis could be regulated by a such proportional control scheme with different thermal sensitivities emanating from different thermal inputs. Several models predict metabolic response to cold using such a proportional control scheme from measurements of mean skin temperature and internal core temperature (T_c) in which there is a linear dependence of these two variables (Gagge and Gonzalez, 1996; Hammel,

1968). Others (Stolwijk and Hardy, 1997; Tikuisis et al., 1991) have shown that shivering thermogenesis is closely associated with thermal signals from both the body core and mean weighted skin sites and heavily influenced by %body fat.

Evidence from this study indicates that along with proportional control there is a certain level of rate control that likely contributes to the shivering responses operating during rest in the two menstrual cycle phases. We showed in the transient analyses that skin and esophageal temperatures primarily contribute as additive terms to the total shivering response. Finger temperature (e.g., thermal inputs from acral areas) is multiplicative with total control of the shivering response.

Skin heat flux responses. The weighted heat flux obtained in these experiments represents an independent assessment of cutaneous blood flow stemming from both extremity (arms,thighs, and calf) and chest skin surface sites purported to be responsive to neural efferent drive controlling skin blood flow (Ducharme et al., 1990; Gagge and Gonzalez, 1996). If cutaneous heat flux reflects the concomitant effect of augmented peripheral blood flow throughout the body's system, which we believe it certainly does, then the mean weighted heat flux responses found in the present study (Figure 4) definitely concur with studies showing increased arm blood flow by direct vasodilatory action or attenuation of vasoconstrictor activity prevalent in the luteal phase of a woman's menstrual cycle (Bartelink, et al., 1990; Hassan, et al., 1987).

During experiments in the luteal phase, in which the women wore Ensemble A, increases in mean weighted heat flux from control period were initiated earlier and rose to a much higher level at the 40, 60 and 80th min time points of the cold transient

(Figure 4) compared to the experiments during the follicular phase ($P<0.05$). There occurred a decreased level of cutaneous heat flux evident with Ensemble B during the cold transient. This would be a requirement owing to the summed effect of increased tissue resistance during vasoconstriction plus trapped heat flow owing to a higher thermal resistance provided by the extra layers of clothing. This combination likely caused a reduction in the skin to ambient temperature gradient, thereby constraining skin heat flow to the ambient.

The increased heat flux responses observed in the luteal phase with ensemble A are at variance with steady-state studies of women exposed to 90 min of a 28°C ambient temperature showing decreased thermal conductance in the mid-luteal phase (Frascarolo et al., 1990) and other resting studies showing reductions in thermal conductances (Bittel and Henane, 1975). These latter studies proposed that the offset in core temperature apparent in the luteal phases of women is the outcome of an increase in tissue thermal insulation. Our data suggest, on the other hand, that such observations of reliance on thermal insulation per se are probably not in effect when peripheral cold receptor activity (e.g., dynamic skin receptors) dominates in resting clothed women.

The elevated heat flux response displayed in the luteal phase with Ensemble A suggests that the response results from augmented endogenous levels of estradiol and/ or progesterone acting centrally (Boulant, 1996) or in peripheral vascular sites (Bartelink et al., 1990; Hassan et al., 1987). In one study (Hassan et al., 1987), lowering of the foot below heart level induced vasoconstrictor responses on women resting supine in a neutral thermal environment. These responses were strongly influenced by hormonal level at two stages of the menstrual cycle. During the follicular phase (low estradiol and progesterone),

postural vasoconstriction was at its maximum. Alternatively, in the luteal phase (high estradiol and progesterone) the postural vasoconstrictor effect was significantly reduced and the women exhibited higher skin blood flows (laser Doppler flowmeter) than found in the follicular phase experiments.

In our study, it was not possible from our experimental techniques to deduce whether the heat flux response derives from a direct CNS response activating a peripheral vasodilatory reponse or occurs primarily via release of vasoconstrictor response at various peripheral vascular sites (Bartelink et al., 1990). The effect of augmented skin heat flux, and delayed latency in time for its initiation, during the luteal phase implies a general neural controlled response clearly modulated by the two reproductive hormones.

Body heat debt. During resting states, whole body exposure in the cold is limited by time, ambient temperature, and wind speed (Gagge and Gonzalez, 1996; Tikuisis et al., 1991; Vallerand et al., 1992) and the body often loses heat faster than it can produce it. The lower limits of a person's ability to regulate internal body temperature are set when the metabolic heat flowing to the skin surface can no longer equal the heat loss from the skin surface. As pointed out previously, these limits can be extended by addition of clothing insulation which adds a finite thermal resistance layer to the skin layer and this property along with body fat act to extend cold tolerance limits (Fig 2 and 3).

Analysis of rate of heat storage in the heat balance equation (in this case, -S or heat debt) (Gagge and Gonzalez, 1996; Vallerand et al., 1992) (Figure 5) provided a highly valuable indicator of the summed effects of cold stress. Our data show that, when the

tissue resistance (skin+body fat) and thermal resistance by clothing is constant and low, sensible heat loss and shivering thermogenesis are uniquely linked in the two disparate stages of the menstrual cycle. Shivering thermogenesis, strongly under hormonal control, may respond with a time constant slower than the neural control activating sensible heat loss.

Decreases in rate of heat debt were readily apparent following 40min of the cold stress at which time point there occurred a definite separation of sensible heat and shivering thermogenesis (Fig 5). Apparently, in the follicular phase with prolonged inactivity, sufficient heat cannot be generated to prevent excessive body cooling when wearing an ensemble having the thermal resistance equivalent to street clothing (Ensemble A). On the other hand, the enhanced thermogenic activity observed during the luteal phase, plus added thermal resistance amounting to street clothing, possibly operate analogously to a heat generator in open air. In the luteal phase experiments with Ensembe A, rate of heat debt was significantly less in the women compared to the follicular phase at time periods from 40min to the 80th min of exposure with Ensemble A. Body heat debt in the luteal phase was also less at 40,60 and 80th mins ($P < 0.05$, 3way ANOVA with Tukey HSD post hoc) than that observed in both the follicular and luteal phases with Ensemble B. However, ensemble B with a higher thermal resistance than ensemble A also has outer semipermeable layers which impede insensible heat loss. Vapor accumulation potentially causes condensation within the layers lowering the intrinsic insulation and increasing skin wettedness (Gagge and Gonzalez, 1996). These factors may have contributed to elevated heat debt evident with Ensemble B during the final time points of the cold transient. During the luteal phase when the women were dressed in ensemble A,

sensible heat produced was also impeded by the intrinsic insulation of the ensemble higher than bare skin. However, this ensemble is vapor permeable, and without moisture condensation, any trapped heat apparently was sufficient to prevent excessive body cooling during the transient.

Control mechanisms affected by central action or influence by reproductive hormones

The results of changes in shivering thermogenesis, heat flux, and heat debt presented in this study favor the hypothesis that total mobilization of responses are potentially influenced by either the singular or multiple action of reproductive hormones. These may act systemically or directly on the thermoregulatory center. Various substances have been implicated which act as neuromodulators, altering the cellular dynamics of thermosensitive neurons in the hypothalamus (Boulant, 1996; Cannon and Dinarello, 1985; Scott et al., 1987). These neuromodulators have been shown to also trigger various efferent thermoregulatory responses.

Several reports have shown (Boulant, 1996; Hammel, 1968; Nakayama et al.,1975) that preoptic/anterior hypothalamic stimulation of warm sensitive neurons results in reciprocal inhibition of cold sensitive neurons mediating an active vasoconstrictor response that facilitates cutaneous heat loss. Additionally, many of these thermosensitive neurons are very responsive to reproductive steroids. Estradiol administration (30pg/ml) in brain slices from male rats (which have no connection with peripheral afferents) preferentially stimulated warm sensitive neurons which increase their firing rate and generally influence heat loss responses. Excitation of warm sensitive neurons have been shown to also inhibit cold sensitive neurons that drive heat production responses (thereby decreasing

shivering thermogenesis) and heat retention responses (attentuating cutaneous vasoconstrictor control). The whole body outcome of elevated estradiol acting singularly on the hypothalamic area, assuming zero or neutral drive from skin receptors, would be to deter shivering and increase vasodilation by inhibition of cutanteous vasoconstrictor activity. Nakayama et al.(1975) found that systemic injections (i.m. and i.v.) of progesterone primarily decreased the rate of firing of warm sensitive neurons but stimulated cold sensitive neurons in intact female rabbits exposed to room temperture. Nakayama et al.(1975) suggested that the decreased activity of warm sensitive neurons, and inibition of cold sensitive neurons by singular action of progesterone, would result in an upward alteration in the thermal reference point. The whole body response outcome (not evaluated by Nakayama et al.,however) in a neutral thermal environment would be to elicit an elevated shivering response and concomitant vasoconstrictor response.

Cannon and Dinarello (1985) first showed that plasma bioactivity for IL-1 is higher for women during the luteal phase than for men or women during the follicular phase. Recently, Cannon found that IL-1β activation was positively correlated with plasma progesterone but negatively correlated with urinary estradiol concentration (Cannon, personal communication). Such findings would appear to show that a tight linkage of progesterone and IL-1β may accompany (or precipitate) some of the thermogenic effect responsible for internal body temperature rises. Conceivably, these cytokines may alter the cellular dynamics inherent in warm, cold, or temperature insensitive neurons controlling thermal signals from the anterior hypothalamus (Boulant, 1996). Then again, cytokines may not participate as significant modulators in healthy eumenorrheic women except possibly during excessive physical activity or during the acute phase response active

during systemic infection. Recently, Rogers and Baker (1997) showed that synthetic progestrins in oral contraceptives altered the thresholds for heat loss in women during rest and exercise. However, they found that plasma levels of the cytokines IL-6 and IL-1β were not potential inducers of this change in core temperature response since a cohort of women not taking oral contraceptives exhibited no significant changes in the plasma levels of these cytokines nor alterations in body fluid parameters.

In respect to our results, an attractive paradigm centers on the role attributed to either estradiol or progesterone in the luteal phase, acting in concert or singularly, inducing appropriate physiological changes during the offset towards a higher body temperature of eumenorrheic women (and possibly even those women taking synthetic progestrins). The general results of our study parallels Boulant's (1996) model and Hammel's original concept (1968) that activation of warm sensitive neurons is accompanied by reciprocal inhibition of cold sensitive neurons. Following excessive cold perturbations, these two reproductive steroids may likely trigger heightened activity in warm sensitive neurons in the preoptic/anterior hypothalamus. Activation of warm sensitive neurons, along with reciprocal action via interneurons, conceivably might render a dampening of cold-sensitive neurons. Both direct and indirect actions would modify heat production responses (reduced slope of $\Delta M/\Delta \bar{T}_{b,i}$) and constrain heat conservation effector drives (release of vasoconstriction). In our study (Figures 3,4, & 5; Table 2), when the women were dressed in an ensemble equivalent to street clothing, the attenuation of shivering thermogenesis per integrated mean body temperature ($\bar{T}_{b,i}$) in the luteal phase was accompanied by an excess mean weighted heat flux throughout the body and a blunted rate of heat debt. These results coincide with the general supposition that hypothalamic changes (Boulant,

1996; Nakayama, 1975), possibly due to the elevated estradiol and progesterone blood levels in the luteal phase of a woman's menstrual cycle, may well activate appropriate efferent responses. However, extensive interaction (and possible competition) between thermal inputs from skin and deep body thermoreceptors probably exists tempering the final summed shivering responses in women as a function of the menstrual cycle.

In summary during the resting phase, effects of the menstrual cycle on heat loss and heat production (M), core (T_c) , and skin temperature responses were studied in six unacclimatized, women nonsmokers (ages 18-29 yr), resting supine. Each women was exposed to a cold ramp ($T_a = \bar{T}_r$ = 20°C to -5°C, -0.32 °C/min, rh 50 ±2%,V=1m•s^{-1}) in the follicular phase (F=days 2-6) and mid luteal phase (L= days 19-23) of her menstrual cycle. It was shown that extensive peripheral vasoconstriction in F during early periods of the ramp generally elevated resting T_{es} and $\Delta \bar{T}_{es}$ above thermoneutral levels. Shivering thermogenesis ($\Delta M = M - M_{basal}$, W•m^{-2}) was highly correlated with ($\downarrow \bar{T}_{sk}$), and ($\downarrow T_{fing}$) ($P<0.0001$). There was a reduced slope in the ΔM as a function of ($\bar{T}_{b,i}$) in L experiments when dressed in BDUs ($P<0.02$) and BDU+BDOs ($P<0.01$). HF was higher and -S was less in the L phases when dressed in BDUs ($P<0.05$).

One of the priorities for future research should be in further quantification of the role of cytokines and reproductive hormone fluctuations on thermoregulatory control in women at various stages of the menstrual cycle during cold stress. Especially needed is information on shivering thermogenesis in women at various stages of the menstrual cycle perturbed by lowered core temperature (-0.2 to - 0.5°C from neutral) with dynamic skin temperature decreases.

B. **Whole body thermoregulatory model developed for cold-air stress resting experiments**

Since 1983, specific attention has been directed toward the modeling requirements of individuals working in temperate and warm environments. Little efforts have been focused on ways to characterize physiological mechanisms during cold stress in women and relate these to an acceptable thermoregulatory model formulation. This part of the project was a preliminary attempt to model women responses to cold stress. Prediction of shivering thermogenesis by using the various thermoregulatory model equations has not specifically focused on explaining responses owing to the luteal phase offset in internal body temperature (Kolka and Stephenson,1995). Additionally, many of these models to date have not fully incorporated responses due to the separate influences of cold fingers and cold toes (acral drives). During cold weather field operations, especially at rest and light activity levels, the hands and feet are the probable locations of thermal discomfort, local cold injury, and loss of sensation or dexterity which affects performance. We found that the skin temperatures and (skin blood flow) of finger and toes are disproportionately important in terms of maintaining thermal comfort, improving morale and performance. Since it is impossible to obtain experimental data for all possible combinations of clothing, handwear and environmental conditions, a model, based on experimental data, for predicting soldier endurance times in the cold has considerable military significance.

<u>CIVD Observations</u>

One model (Shitzer et al., 1996) in this project was developed by a Senior NRC fellow funded by the proposal. His model predicts the maximum endurance times for an

individual based on calculated values for finger temperatures. The model is based on a scenario with and without cold-induced vasodilation (CIVD) response and predicts blood flow to the extremities during the response. Additions needed to the model require morphological features of finger and hand sizes and autonomic control characteristics expanded to females since we have shown in this study that there is a differential response in cutaneous heat loss during the various stages of the menstrual cycle in females. For example, from our resting study results, It can be deduced that augmented hormonal levels (elevated progesterone and/or estradiol) in the luteal phase may interact with local chemical mediators (e.g., nitric oxide and/or adenine) modifying α_2-adrenoceptors. In the luteal phase there could be a blunted delay in the ability of norepinephrine released at specific vascular sites to induce contraction of smooth muscle at cold temperatures (Flavahan, 1991; Furchgott, 1993; Hassan and Tooke, 1987; Koenig et al., 1995). The observations in the resting study suggest further experiments on intact extremity sites or using isolated blood vessels (animal model using both genders) to elucidate mechanisms of response characterizing differential effects of estradiol or progesterone blood levels on arteriovenous-anastamoses (AVAs) receptors during CIVD.

The crux of the project to develop a simulation technique useful in forecasting women response to cold stress was very fruitful but still remains the focus of additional work not funded from this project. Past thermoregulatory models have incorporated shivering algorithms, primarily applicable for unclothed men, determined as a function of core and mean skin temperature response (Stolwijk-Hardy, 1977; Wissler, 1985). Some recent progress has been accomplished toward factoring other variables such as % body fat, lean body mass, and rate of change of skin temperature (Tikuisis and Gonzalez, 1988; Tikuisis

et al., 1988; Tikuisis et al., 1991; Hayward et al., 1977; Timbal, 1976). Table 4 shows algorithms examined from some of these cold stress prediction equations that are pertinent to compare with the results from the resting part of the study.

Figure 8 shows the prediction of shivering thermogenesis from the various models applied to the specific body temperatures observed during cold transient of the present study. All prediction models have been normalized to reflect ΔM (in $W \cdot m^{-2}$) as previously described (Tikuisis et al.1988). Our data suggest that temperatures from acral sites, independent from a given core site and a mean weighted skin temperature ($\bar{T}_{sk}$), should definitely be considered in any whole body thermoregulatory models describing $\triangle M$ effects in resting women (and presumably in men also). This response was adequately simulated by use of parameter estimation analysis confirming that finger temperature is a significant amplifying factor in any cold shivering thermogensis along with %BF (which is the primary attenuator of the response), and lean body mass (Buskirk et al.,1963; Tikuisis et al., 1991; Timbal, 1977). Early studies by Carlson and others (Carlson, et al., 1970) in men alluded to the effect of local finger temperature having a critical effect on shivering thermogenesis. Studies on various animal species have concurred with this conclusion (Gonzalez et al., 1971; Simon,et al., 1986). Interestingly, the rabbit ear has been shown to be a reliable analogue of skin blood responses occurring in the human hand as well (Gonzalez, et al. 1971).

Figure 8 shows that of the four whole body algorithms predicting shivering thermogenesis as a function of minutes of the present cold ramp, determined exclusively from a cold stress database in unclothed men, the Stolwijk-Hardy equation (1977) proved the most over predictive. However, the models formulated by Tikuisis et al. 1991 (%BF),

Timbal (rate of change of skin temperature), and Hayward's (multiplier of core and skin temperatures from a thermal convergence component) were very predictive of the responses observed in women dressed in BDUs during both phases of the menstrual cycle. This preliminary observation suggests that during mild drops in core body temperature either of the latter three cold-air model algorithms may be adequately utilized as acceptable operational indices (but not mechanistic indicators) of excess shivering response in both males and females.

All the above models and the present study's algorithm were compared to data from an independent study of men and women exposed to steady-state cold stress of 5°C (Graham et al., 1989). Graham studied eumenorrheic, amenorrheic, and men dressed in PT clothing exposed for one hour in the cold during rest and exercise. Figure 9 shows the metabolic heat production data (normalized to ΔM, $W \cdot m^{-2}$) from Graham and coworker's study compared to the various cold-air model predictions using results from the eumenorrheic women (%BF= 21.5 %) and men (%BF=8.9%). Rectal temperature was used in place of the various core temperature sites of the various models.

The shivering thermogenesis data observed from the women predicted very adequately up to the first 50 min of steady-state cold stress using the different models (including the Stolwijk-Hardy algorithm) with a combined regression coefficient of determination, r^2=0.86. Observations from the male data in Graham's study were less predictive (r^2 = 0.65) of the data using the various models. The highest predictors of the data were the following: the Stolwijk-Hardy algorithm, the Tikuisis' model which

incorporates %BF in its development, Timbal's algorithm, and the present maximum likelihood estimation (MLE) prediction. In the latter MLE equation, toe temperature recorded from the Graham paper was used in lieu of finger temperature in the model. Eumenorrheic women were assumed to be in the follicular phase.

For a combined consolidated thermal model, the Tikuisis (1991) equation appears to be a suitable first approximation strategy that can be used for depicting shivering thermogenesis if peripheral (finger) temperatures cannot be determined as input to model shivering thermogenesis. The algorithm is especially useful in predicting ΔM provided the dominant thermal drive is not from decreasing core temperature and individuals are within a range of %BF between $\geq 8\% \leq 30\%$.

C. Effects of the menstrual cycle during cold stress and exercise: cytokine interaction and cardiorespiratory responses

Strong evidence ties the immune and reproductive systems demonstrating that cytokine elevations during moderate exercise may influence the pattern of menstrual response and endocrine bio-rhythms (Cannon, 1993; Northern et al., 1994). Core temperature rises coincide with increased blood levels of 17β estradiol (E2) and progesterone (P4) in the mid luteal phase (L) of a women's menstrual cycle (Kolka et al., 1997). It has been postulated that there is a set point change in the CNS thermostat owing to the activation of the E2 and/or P4 or cytokine activity, particularly IL-1β (Cannon and Dinarello, 1985).

This part of the study was done to characterize the effect of exercise during the imposition of a continuous cold stress while women exercised dressed in a BDU+Parka. Thermoregulatory responses were followed in nine healthy women with regular menstrual cycles (means ±SD: age,26.3 ±4.6 y;54.9 ±4.0 kg;height 1.62 ±0.05m; $\dot{V}O_2max$ = 46.2 ±4.8mL•kg^{-1}•min^{-1}). Each woman served as her own control as she was exposed to a cold challenge (T_a) as in the resting experiments during their early follicular (F) and mid luteal (ML) phases. The women were exposed to cold stress while walking on a treadmill at a work rate of 32 % $\dot{V}O_2max$ dressed in BDUs + parka. T_a was dropped from 20.0 °C to -5.0 °C (-0.3 °C/min) over 80 min which incurred sufficient skin cooling to create an adrenergic response due to extremity discomfort but did not reduce any of the effects of endogenous thermal input from exercise on control of sweating and skin blood flow. As in the resting studies, esophageal (T_{es}) and skin ($\bar{T}_{sk}$) were measured continuously. Arm sweating rate ($\dot{m}_s$) and regional skin heat flow ($\dot{H}_{sk}$) were measured continuously as well

during the exercise. Cardiac output ($\dot{Q}$), $\dot{V}_{O2}$, $\dot{V}_{CO2}$, and $\dot{V}_E$, were measured periodically until T_a reached 5°C. Subjects were clothed in BDUs+ Parka along with current issue combat boots and work gloves. The 9 women volunteers were studied using a repeated measures design. $\dot{V}_{O2}$ were recorded using a SensorMedics Metabolic cart. Cardiac Output ($\dot{Q}$) was measured using a non-invasive CO_2 rebreathing technique (Heigenhauser and Jones, 1974;Hatcher and SRB,1986). Mean skin temperature was determined by area weighting of five site skin temperatures plus finger temperature and big toe temperature, esophageal temperature, and heat flow using calibrated heat flow disks (Concept Eng, Hartford,CT) at the same sites as where skin temperature is recorded. Sweating rate was continuously recorded using an automated dew point sensor method (Graichen et al.,1982)

Venous blood samples were collected prior to, 30min during, and immediately post exercise for determination of serum estradiol (E2) and progesterone (P4) by radioimmunoassay; additionally, IL-1β, IL-6, and tumor necrosis factor (TNF) activity were analyzed by immunoassay (Capper et al., 1990; Cannon et al., 1993; Kluger et al., 1995; Leon et al., 1997). The data were analysed by ANOVA (experimental variable by time) with repeated measures. Tukey's test of critical differences was performed as a *post hoc* analysis of a given parameter ($P < 0.05$). Paired t-tests were used as appropriate to compare the pre and post exercise differences in cytokine and physiological variables.

Results

In these nine women, E2 levels were :(mean±1SD) 30.08 ±10.38 and 80.9 ±18.46 $pg \cdot mL^{-1}$, while P4 levels were 3.04±1.03 and 29.3±13.6 ng •mL during the F and ML phases post exercise ($P<0.05$ time and phase) respectively. Luteinizing hormone (LH) was constant (2.74±0.95 F and 2.17±1.62 $mIU \cdot mL^{-1}$) in each phase. A higher offset in T_{es} (P

< 0.03) during the mid luteal phase and persistent separation between the cycle phases was observed throughout exercise. During the first 40 min of exercise/cold stress, $\dot{m}_s$ and $\dot{H}_{sk}$ increased as a $f(T_{es})$, then became inhibited by $-\Delta\bar{T}_{sk}/\Delta t$ ($P<0.05$).

It was found that T_{es} and $\dot{m}_s$ during ML remained elevated in the women ($P<0.03$ by cycle phase; $P<0.0001$ by time, Figure 6). The $\Delta \dot{m}_s/\Delta T_{es}$ was higher in the ML phase compared to the F of each women throughout the exercise. The exercise/ cold stress (e.g.,a non-infectious adaptive response) triggered minimal perturbations in the cytokine activity (Figure 7). IL1-β, IL-6, and TNF were not significantly increased in either of the women's cycle phase. In the ML cycle phase, IL1-β was decreased post exercise by 41% ($P<0.02$) from pre-exercise value.

In both cycle phases during the cold, $\dot{V}_E$ was highly correlated with $\dot{V}_{CO2}$, and T_{es}, but negatively correlated with $\bar{T}_{sk}$. $\Delta\dot{V}_E/\Delta T_{es}$ was decreased in the luteal phase (-64%). $\dot{Q}$ was highly correlated with $\dot{V}_{O2}$, $\dot{V}_{CO2}$,T_{es} and negatively correlated with $\bar{T}_{sk}$. Slopes of $\dot{Q}$-$\dot{V}_{O2}$, $\dot{Q}$-$\dot{V}_{CO2}$ and $\dot{Q}$-T_{es} were decreased in L during the cold transient with exercise (Table 4).

DISCUSSION

In this part of the project, it was found that sudomotor and H_{sk} responses were possibly activated by surge of estradiol and/or progesterone levels without concomitant increases in specific cytokines. This suggests potential blunting of cytokine activation by these reproductive hormones during exercise and cold transients in the ML phase. In this study, specific cytokines generally appearing as a consequence of acute phase responses (Cannon, 1993; Kluger et al., 1995; Hoffman-Goertz and Pedersen, 1994) were not activate in the healthy eumenorrheic women studied. Recently, Rogers and Baker (1997) compared the thermoregulatory response of seven women to treadmill exercise during the

third week of pill use (P) and the week when no pill was ingested (N). Five of their seven subjects were using a triphasic combination oral contraceptive and two of their subjects were using a monophasic combination pill. Resting core temperature was 0.31°C higher and the threshold for the sweating onset was 0.32°C higher in P than in N, respectively. Their results show that the progestin component of the pill has a dominant effect on thermoregulation (Rogers and Baker, 1997). Plasma levels of IL-6 and IL-1β were not potential inducers of this change in core temperature response since a cohort of women not taking oral contraceptives exhibited no significant changes in the plasma levels of these cytokines nor alterations in body fluid parameters.

An attractive paradigm of action comparable to our study centers on the role attributed to P4 inducing the offset towards a higher body temperature in the mid-luteal phases of eumenorrheic women (and possibly even those women taking synthetic progestrins). Kluger and co-workers' (1995) model of fever suggests that TNF, IL-6 and other cytokines from the viscera are not immediately responsible for initiation of fever. The release of IL-1β which cascades into release and surge of IL-6 possibly raises the internal set point. TNF (circulating and hypothalamic) is actually antipyretic and to an extent, cyrogenic (Leon et al., 1997). The activation of IL1-β, IL-6, and TNF, alternatively, induce release of the antipyretic hormones: corticosterone and arginine vasopressin (AVP). Integrated with this model concept is Boulant's (1996) model of neuronal activity which is a combination of interaction between temperature insensitive (I), warm sensitive (W) and cold sensitive (C) neurons activitating discrete thermoregulatory effector responses. Our study indicates that surge of circulating E2 and P4 in the mid luteal phase of a women's menstrual cycle may also act to modulate the pre-optic set point temperature (T_{po}). Indeed,

a recent study (Griffin and Saper, 1996) shows that IL-1β inhibits activity of hypothalamic warm sensitive neurons mediated by prostaglandins. Since P4 as a Carbon-21 steroid is closely analogous to actions triggered by corticosterone, and E2 activates AVP increasing cyclic AMP (Kluger et al., 1995), these hormones likely modulate neuronal activity in the hypothalamus.

Elevations in these hormones could conceivably dampen pyrogenic action of IL-6 induced by IL-1β. The increased cutaneous and sweating rate apparent in the luteal phase, and concomitant inhibition of IL1-β in the luteal phase experiments, suggest that the immune system and endocrine system are tightly connected. It has been shown that estradiol induces a protective action on the vascular system during nitric oxide synthesis (Senie et al., 1991). Additionally, some clinical trials indicate that overall and recurrence-free survival rates of women with operable breast cancer were markedly enhanced in the women undergoing surgery in the mid luteal phases when the surge in E2/P4 is augmented (Northern et al., 1994; Senie et al., 1991). One mechanism operative in the acute phase cascade response (Cannon, 1993) that deserves closer examination by further parallel studies is the effect of moderate exercise in the cold with the occurrence of a long-term adaptation response. Possibly progesterone-induced changes during moderate exercise might trigger the synthesis of the 70-kDa family of heat shock proteins (Ganong, 1977). These latter proteins are released prior to initiation of cell damage which possibly inhibit cytokine production from inflammatory cells or hyperthermic neurons (Cannon, 1993; Northern et al., 1994; Boulant, 1996). These substances, as well as endogenous E2/P4 during heavy exercise may behave as potential homeostatic enablers active during a woman's mid luteal phase in prevention of injury whenever there is a

resetting of the core temperature. In this respect, E2/P4 can serve as critical couplers with the immune and thermoregulatory systems. Another interesting study would be to investigate the pattern of heat conservation and heat loss responses tied in with continuous measurements of body temperatures, specific cytokines, and E2/P4 release occurring during sleep in women at variable stages of the menstrual cycle when it is known that T_{po} and plasma cortisol become reduced (Kluger, 1995). The cardiorespiratory results (Table 3) suggest that during submaximal exercise/cold stress, control of ventilation and cardiac output may be linked with altered T_c but the heat exchange (sudomotor and skin blood flow) requirements are driven by thermoregulatory adjustments arising during alterations of reproductive hormones throughout a woman's menstrual cycle. On the whole, during submaximal work in cold transients, effect of the menstrual cycle did not appreciably alter the magnitude of cardiorespiratory adjustments which were linked to percentage of maximal aerobic power (Åstrand et al., 1964; Dombovy et al., 1987; Jurkowski et al., 1981). Cardiac output response in this study emerged primarily as a consequence influenced by increased metabolism resulting from thermoregulatory compensations attempting to maintain internal body temperature to cold+ exercise load. These results are in general confirmation with other studies in which specific ventilatory parameters, albeit affected by the menstrual cycle, do not influence submaximal exercise performance (Dombovy et al., 1987; Astrand et al., 1964). A more thorough model parameter sensitivity analysis comparing central cardiovascular parameters (stroke volume, cardiac output, cardiac frequency, etc) is necessary to fully verify model algorithms for heavy and maximal exercise in women. Based on our preliminary results, present thermoregulatory-based stochastic models appear to be reasonable predictors of cardiorespiratory parameters for

both male and female responses to cold stress during light to moderate exercise.

CONCLUSIONS

1). An analytic model developed from the proposal results revealed that $\bar{T}_{sk}$ and T_{es} contribute as additive inputs and T_{fing} has a multiplicative effect to the total control of ΔM during cold transients (R^2=0.9). Endogenous hormonal levels at each menstrual cycle phase,T_c and $\bar{T}_{sk}$ inputs, vascular responses, and variations in body heat balance must all be considered in quantifying thermoregulatory responses in women during cold stress. During cold transient stress in resting women at two stages of the menstrual cycle: a) A decreased slope in the luteal phase was observed when shivering thermogenesis was plotted as a function of integrated mean body temperature assessed from heat balance; b) During the luteal phase when heat balance becomes modified by changes in body temperatures, direct and indirect action of enhanced endogenous estradiol and/or progesterone possibly attenuate the peripheral sensitivity (cold skin thermal drives) to central temperature-mediated thermal signals influencing ΔM; c) Metabolic response in both the luteal and follicular phase is correlated with mean skin temperature and internal body temperature, but highly influenced by thermal inputs from acral sites; d)The greater heat flux response observed in the luteal phases confirms results shown before in women relative to skin blood flow responses.

2). The exercise study additionally yielded additional quantitative physiological data on females studied in two stages of the menstrual cycle (follicular and luteal) dressed in Battle Dress Uniforms (BDU)+Parka. An adequate screening test for Cold-Induced Vasodilation (CIVD) was developed that incorporates both skin blood flow (Laser Doppler) and skin temperature response. Currently ongoing is a parallel study to compare an ARIEM

developed lumped- parameter finger tip prediction model for resting females at two stages of the menstrual cycle. In the exercise study, nine female volunteers were exposed to a tightly controlled downward ramp of chamber temperatures from 20°C (68°F) to -15°C (5°F) over 80 minutes during which the women did treadmill exercise at 32 % $\dot{V}O_2$max. Cold stress altered the effect of sweating response to the internal body core temperature drives in the luteal phase and attentuated the sweating response in the follicular phases of a women's menstrual cycle.

3. A significant database of resting and exercise data is available for development of algorithms and testing of various male-based cold models useful in development of cold stress guidelines. These data should provide additional information characterizing cold reactions in women at two stages of the menstrual cycle and useful for wider soldier system simulation strategies.

Human subjects participated in these studies after giving their free and informed voluntary consent. United States Investigators adhered to U.S. Army Medical Research and Materiel Command (USAMRMC) Regulation 70-25 on Use of Volunteers in Research.

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APPENDICES

QUANTIFICATION OF RATE OF HEAT STORAGE AND INTEGRATED MEAN BODY TEMPERATURE DURING COLD/RESTING EXPERIEMENTS.

In the cold, initial mean body temperature, $\bar{T}'_b$, is often calculated from a steady-state weighting ratio of mean skin temperature to rectal temperature $\{\bar{T}_{sk} : T_{re}\}$ as 1:2 (Burton, 1935). In the heat or during exercise, the probable ratio varies from 1:4 to 1:9 when esophageal or rectal temperature is used as measure of core temperature (Gagge and Gonzalez, 1996; Stolwijk and Hardy, 1977). During exercise or ambient temperature transients when body temperatures are in a non-steady state, coefficients for mean body temperature change appreciably (Gagge and Gonzalez, 1996; Livingstone, 1967). The calculation of the classical Burton (1935) mean body temperature by a simple weighting of core and skin temperature is inaccurate during thermal transients and probably not applicable in women, since both time and ambient temperature, as well as skin and core temperature vary.

Rate of storage of body heat (*S*), evaluated by partitional calorimetry (Bittel and Henane, 1975; Vallerand et al., 1992; Gagge and Gonzalez, 1996), is directly associated with the rate of change of integrated (i.e., weighted signals from both peripheral and central thermoreceptors) mean body temperature ($\Delta\bar{T}_b/\Delta t$). This form was used in the present study to quantify responses during the cold ramps, in which

$$\pm S = (\lambda \bullet m_b / A_D) \bullet \Delta\bar{T}_b/\Delta t \quad ,W \bullet m^{-2} \qquad (A1)$$

where λ is the specific heat of the body in $0.965 W \bullet h \bullet °C^{-1} \bullet kg^{-1}$
(or $3.49 kJ \bullet °C^{-1}\ kg^{-1}$) and m_b is the body weight in kg; Δt is time in hours.

For this study, during the resting period at T_a = 20°C prior to ambient temperature drops, initial mean body temperature, $\bar{T}'_{b,o}$, was first calculated by a 1:4 weighted average of $\bar{T}_{sk}$ and esophageal (T_{es}) temperature. Integrated mean body temperature (°C) was then determined as

$$\bar{T}_{b,i} = \bar{T}'_{b,o} + \sum_0^t (\Delta\bar{T}_b/\Delta t)\, dt \qquad (A2)$$

or $= \bar{T}'_{b,o} + [(S \bullet A_D)/ (\lambda \bullet m_b)] \bullet \Delta t$ (A2')

where Δt is the interval time ($t_x - t_o$) of a run taken at each $\bar{T}_b$ (min/60) in hours.

Evaluation of integrated mean body temperature at each time point is

$$\Delta \bar{T}_b, i/\Delta t = \frac{[M - (W) - (R+C) - E_{sk}] \bullet A_D}{\lambda \bullet m_b} \quad ,°C \quad (A3)$$

Where λ is the specific heat constant, m_b is body weight, kg, A_D is the DuBois surface area (m^2) and the energy exchange terms in brackets (all in $W \bullet m^{-2}$) are evaluated by partitional calorimetry (Gagge and Gonzalez, 1996).

TABLES

Table 1. Mean (±1SD) concentration levels of estradiol and progesterone in the six women during basal resting periods.

	Estradiol (E2) **($pg \cdot ml^{-1}$)**	**Progesterone (P4)** **($ng \cdot ml^{-1}$)**
Follicular Phase (F) day 2-6	30.2±12.9	0.45±0.24
Luteal Phase (L) days 19-23	122.5±41.1[a]	9.90±4.64[b]

Mean values represent pooled determinations of 12 samples each run in duplicate. Inter assay variability (% differences): L(E2) test 2>test1 by 27%, NS; L(P4) test 2> test 1 by 29%, NS; F(E2) test 1 >test 2 by 14.5%,NS; F(P4) test 1> test 2 by 1%, NS. The six subjects were seated in a 23°C antechamber for 30 minutes dressed in BDUs (ensemble A) prior to experiments. Concentration differences between cycle phase [a]Luteal > Follicular E2 ($P<0.0001$); [b]Luteal > Follicular P4 ($P<0.0001$). Non significant differences (NS).

Table 2. Comparison of slopes of shivering response to $\bar{T}b,i$ and $\bar{T}b,o$, °C during resting cold transient study.

	Slope, $W \cdot m^{-2} \cdot °C^{-1}$	$\bar{T}b,o$, °C
	Ensemble A (BDU)	
Luteal (L)	-23.3±6.8	35.49±0.03
Follicular (F)	-37.1±10.1	35.37±0.18
$\Delta\bar{T}b,o$, °C		0.137±0.19
L<F ($\Delta M/\Delta \bar{T}b,i$)	P<0.02	
$\Delta\bar{T}b,o$, °C		NS
	Ensemble B (BDU+BDO	
Luteal (L)	-20.0±7.5	35.94±0.51
Follicular (F)	-32.9±7.1	35.37±0.18
$\Delta\bar{T}b,o$, °C		0.17±0.20
L<F ($\Delta M/\Delta \bar{T}b,i$)	P<0.01	
$\Delta\bar{T}b,o$, °C		NS

Values are means ±SD. Slopes were obtained from separate piecewise linear regressions in each of 6 women at each cycle phase and ensemble. P value was determined by ANOVA followed by Tukey's honestly significant difference test (SAS, 1995).

Table 3. Algorithms of Various Predictive Models for Cold Stress in the Current Woman Study

†Present algorithms: From resting study	Follicular: $\Delta M\ (W \cdot m^{-2}) = [0.35 \cdot (\bar{T}_{sk} - 32.5) - 0.85 \cdot (T_{es} - 36.9)] \cdot 0.9 \cdot (T_{fing} - 26.5)$ Luteal: $\Delta M\ (W \cdot m^{-2}) = [0.65 \cdot (\bar{T}_{sk} - 31.0) - 2.28 \cdot (T_{es} - 36.9)] \cdot 0.59 \cdot (T_{fing} - 21.5)$
Tikuisis 1991	$\Delta M/lbm = \{0.0422 \cdot (35.36 - \bar{T}_{sk})^2\} / (\%BF)^{0.506}$
Timbal 1976	$MR\ (W \cdot m^{-2}) = 41.31 - 5.01 \cdot (\bar{T}_{sk} - \bar{T}_{sk,o}) - 57.77 \cdot d\bar{T}_{sk}/dt$
Hayward 1977	$MR(W \cdot kg^{-1}) = 0.0356 \cdot (\bar{T}_{sk} - 41.8)(T_{es}^* - 41.0)$
Stolwijk/Hardy1977	$\Delta M\ (W \cdot m^{-2}) = [13(T_{es^*} - 37.0) + 0.4(\bar{T}_{sk} - 34.0)] \cdot (\bar{T}_{sk} - 34.0)$

***T_{es} was substituted for T_{ty} and T_{cor} of original equation parameters; lbm=lean body mass;%BF= per cent body fat. Data for input to the various models were from separate follicular and luteal phase experiments.†from Maximum Likelihood Parameter Estimation (MLE).**

Table 4. Parameter	**Cold Transient on Ventilation during 32%Vo2max Exercise** Follicular Phase	Luteal Phase	**slope L vs F**
$\dot{V}e$-$\dot{V}co2$	$\dot{V}e$= 2.79 +27.65($\dot{V}co2$), r=.97 P<0.05	$\dot{V}e$= 4.07 +27.04($\dot{V}co2$), r=.97, P<0.05	NS
$\dot{V}e$-Tes	$\dot{V}e$= -838.1+22.921(Tes), r = 0.69,P<0.05	$\dot{V}e$ = -288.9 +8.25(Tes), r =0.32, P<0.05	-64%
$\dot{V}e$-$\dot{T}sk$	$\dot{V}e$=166.82-4.784($\dot{T}sk$),r = -.71,P<0.05	$\dot{V}e$=163.5-4.569($\dot{T}sk$), r =-.61;P<0.05	NS
	Cold Transient on Control of Cardiac Parameters during 32%Vo2 Exercise		
$\dot{Q}$-$\dot{V}o2$	$\dot{Q}$= 1.15 + 11.75($\dot{V}o2$),r=.92, P<0.001	$\dot{Q}$= 3.74 + 8.16($\dot{V}o2$), r=.81, P<0.05	-32%
$\dot{Q}$-$\dot{V}co2$	$\dot{Q}$= 1.18 + 13.19($\dot{V}co2$),r = 0.91, P<0.001	$\dot{Q}$= 2.710 + 10.1($\dot{V}co2$), r =.85, P<0.001	-23.4
$\dot{Q}$-Tes	$\dot{Q}$= - 367.1 +10.1(Tes), r=.59, P<0.05	$\dot{Q}$ = -223.6 +6.188(Tes), r =.55, P<0.05	-38.7
$\dot{Q}$-$\dot{T}sk$	$\dot{Q}$= 97.1 -2.853($\dot{T}sk$), r = -.82, P<0.001	$\dot{Q}$= 80.29 - 2.289($\dot{T}sk$), r = - 0.71, P<0.05	NS

FIGURE LEGENDS AND FIGURES

{ACCORDING TO STUDY SEQUENCE}

Fig. 1. Mean (±1SD) ΔT_{es} during minutes of the cold transient in 6 women. Left panel open circles (○) are follicular phase clothed in Ensemble A, (Fol-EnsA); open squares (□) are Luteal phase clothed in Ensemble B, (Lut-Ens A). Right panel closed circles (●) are Follicular phase clothed in Ensemble B (Fol-EnsB); closed squares (■) are Luteal phase clothed in Ensemble B (Lut-Ens B). SD bars are plotted at 10 min intervals for clarity. a= significant difference in ΔT_{es} between phases at each time point (P <0.05).

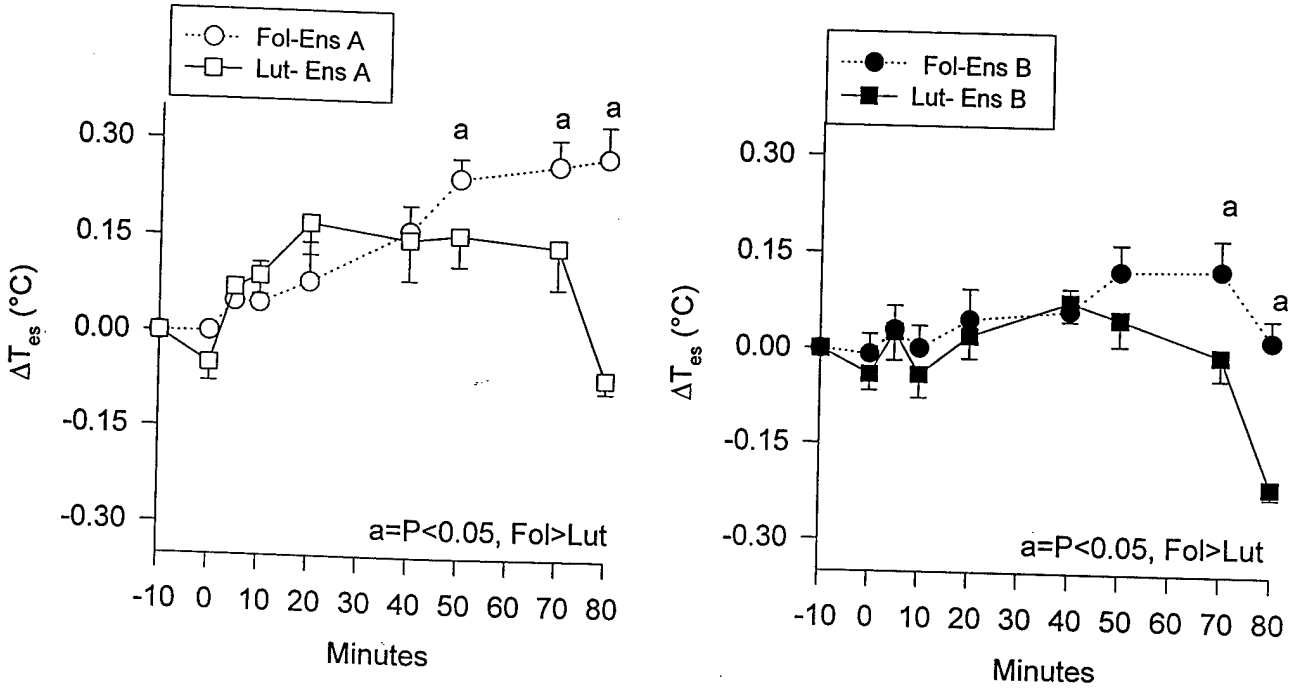


Fig 2. Metabolic heat production (M, $W \cdot m^{-2}$) plotted as a function of $\bar{T}_{sk}$ (linear regression for repeated measures). Top left panel are combined data from women clothed in Ensemble A: Follicular phase,(○), (M= -5.93±0.59 ($\bar{T}_{sk}$) + 227; r^2 = 0.83, P<0.0001); Top right panel Ensemble A: Luteal phase,(□)
(M= -3.93±0.51 ($\bar{T}_{sk}$) + 169, r^2 = 0.76, P<0.0001). Bottom left panel are combined data from women clothed in Ensemble B: Follicular phase, ● (M = -6.56±0.95 ($\bar{T}_{sk}$) +2248; r^2 = 0.66, P<0.0001). Bottom right panel Ensemble B: Luteal,(■) (M= -3.75±0.48($\bar{T}_{sk}$) + 166; r^2 = 0.71, P<0.0001).

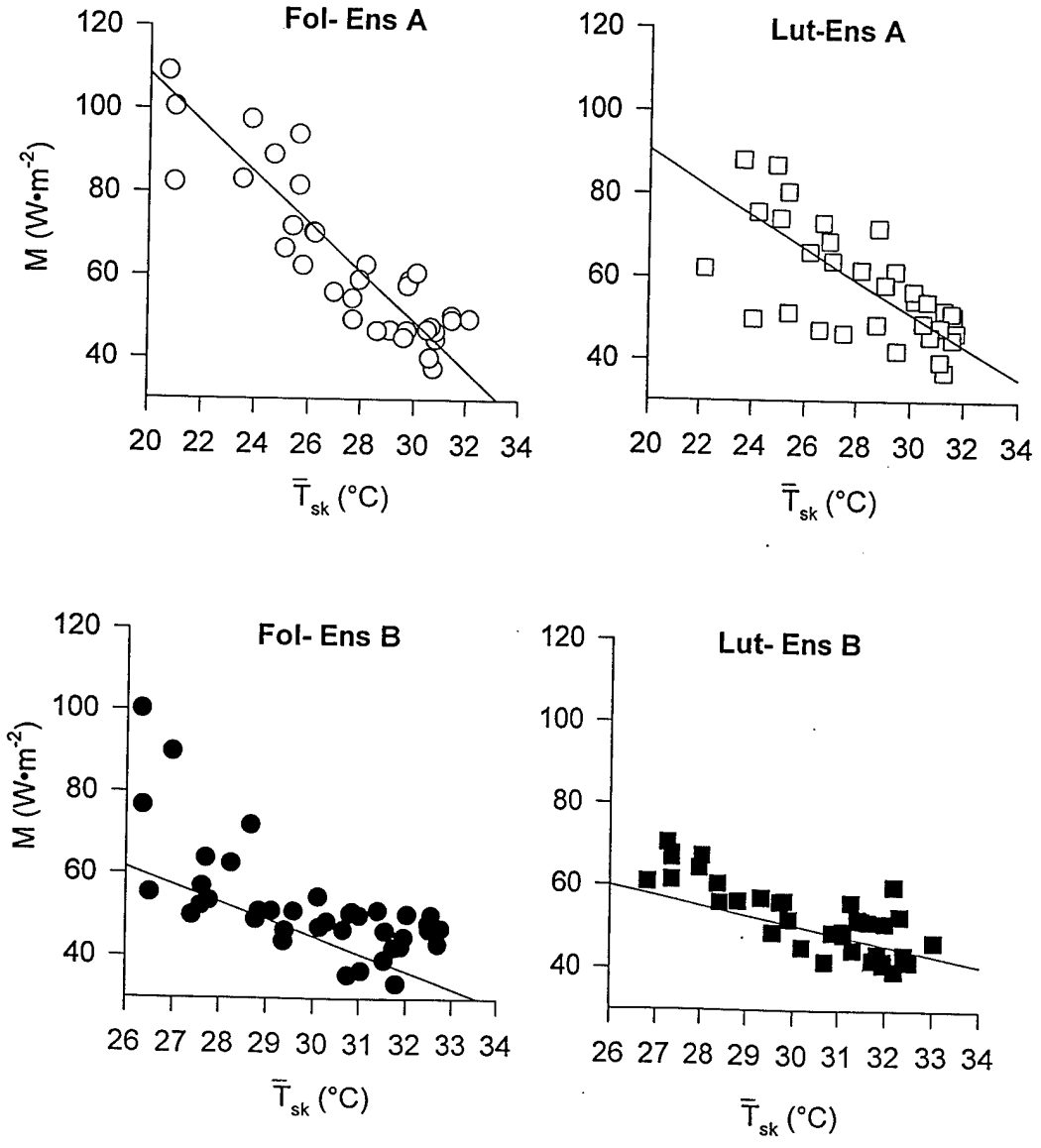


Fig. 3. Metabolic heat production (M, $W \cdot m^{-2}$) plotted as a function of finger temperature (linear regression for repeated measures). Top left panel are combined data from women clothed in Ensemble A: Follicular phase,(○), (M= -1.97±0.21 ($\bar{T}_{fing}$) + 99.2; r^2 = 0.70, P<0.0001); Top right panel Ensemble A: Luteal phase,(□) (M= -1.21±0.12 ($\bar{T}_{fing}$) + 80.3, r^2 = 0.81, P<0.0001). Bottom left panel are combined data from women clothed in Ensemble B: Follicular phase, ● (M = -1.33±0.0.19 ($\bar{T}_{fing}$) +83; r^2 = 0.60, P<0.0001). Bottom right panel Ensemble B: Luteal,(■) (M= -0.75±0.11($\bar{T}_{fing}$) + 68; r^2 = 0.60, P<0.0001).

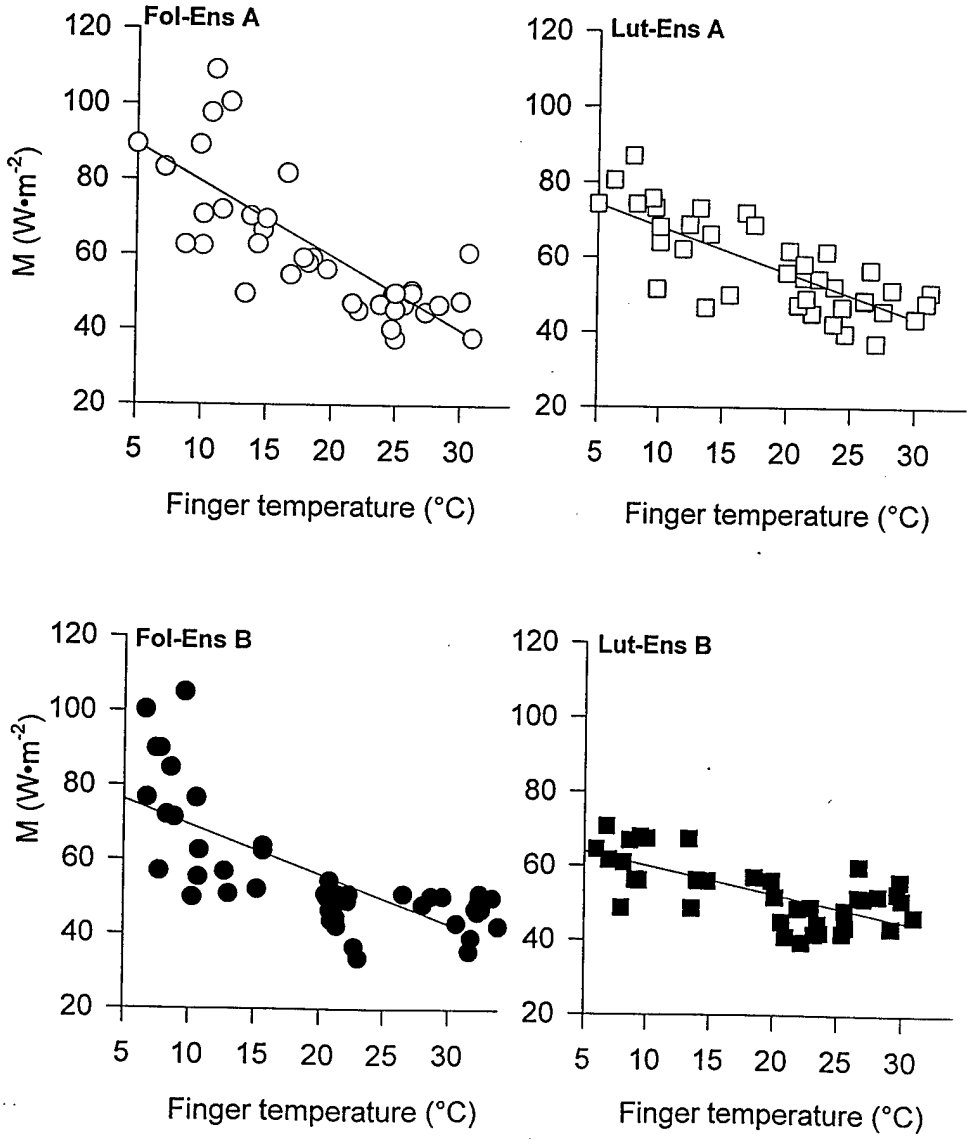


Fig. 4. Mean weighted heat flux ($W \cdot m^{-2}$) ±SD plotted every 20 minutes from time 0 of the cold transient at respective experiments. Symbols coded as in previous figures. *=Significant difference in mean rate of heat flux from baseline values at each given time point (P <0.05); a=Significant difference phases, ensemble A (P < 0.05);b=Luteal > Follicular phase between ensembles (P<0.05). NS= no significant differences between phases, Ensemble B.

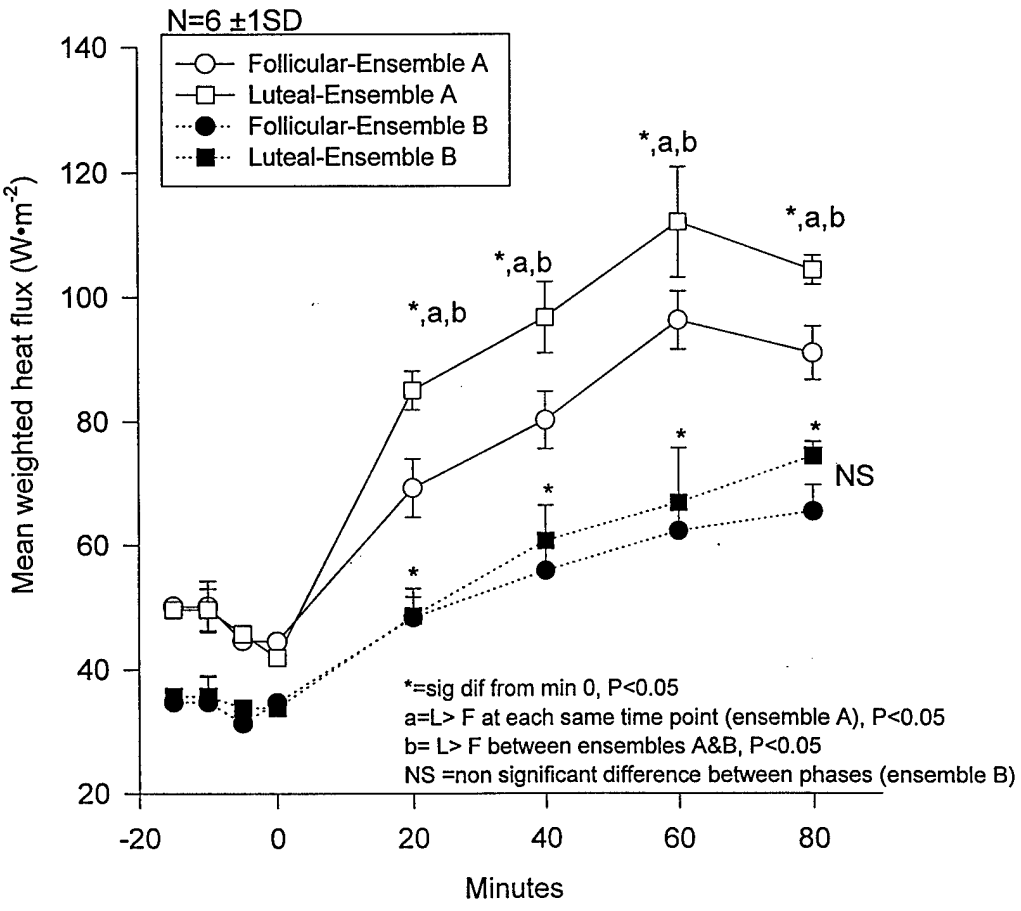


Fig. 5. Mean values ±SD of rate of heat debt (kJ) determined by partitional calorimetry as a function of minutes of the cold transients. Symbols as in the previous figures. *=Significant difference in mean rate of heat debt between from min 0; a= Follicular phase > Luteal phase rate of heat debt with Ensemble A (P<0.05). NS = no significance differences in heat debt between phases, Ensemble B.

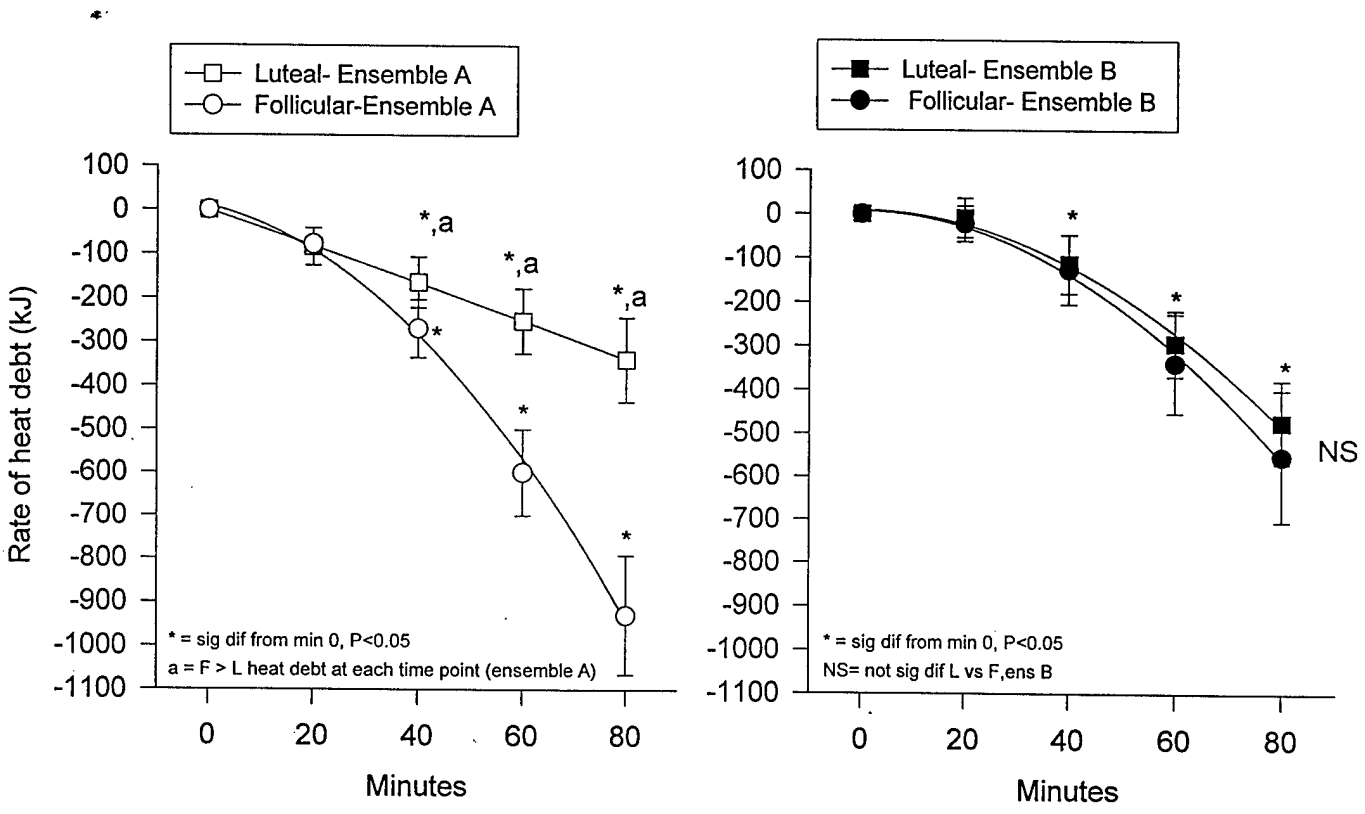


Fig 6. Mean values ±1SD of esophageal temperature during 32%$\dot{V}O_2$ exercise in the cold in nine women at two stages of their menstrual cycle.

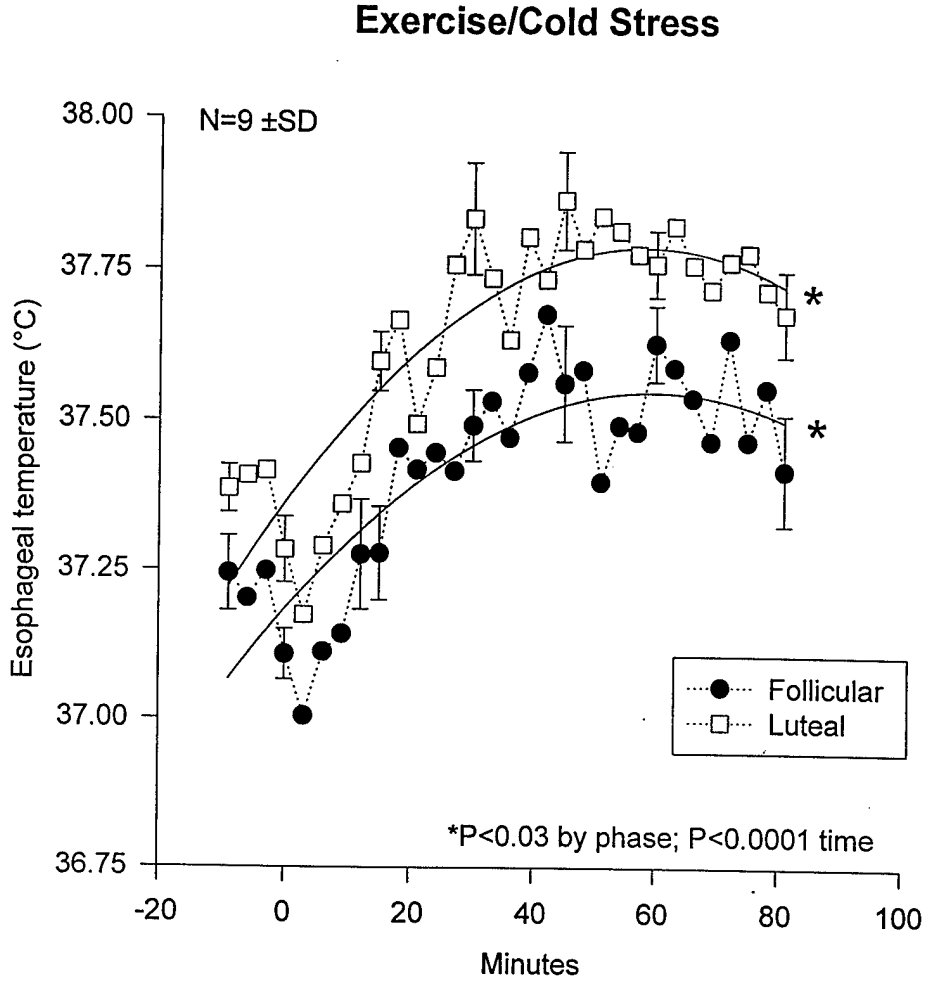


Fig 7. Cytokines (interleukin-6, IL-6; tumor necrosis factor, TNF; and IL-1ß) and sweating rate (ms) during exercise and cold stress in 9 women (means ±SE). Early Follicular (●) and mid-luteal (□) phases. *P<0.05 between phases for time period designated by horizontal bar; **P<0.02 from beginning of exercise to final period.

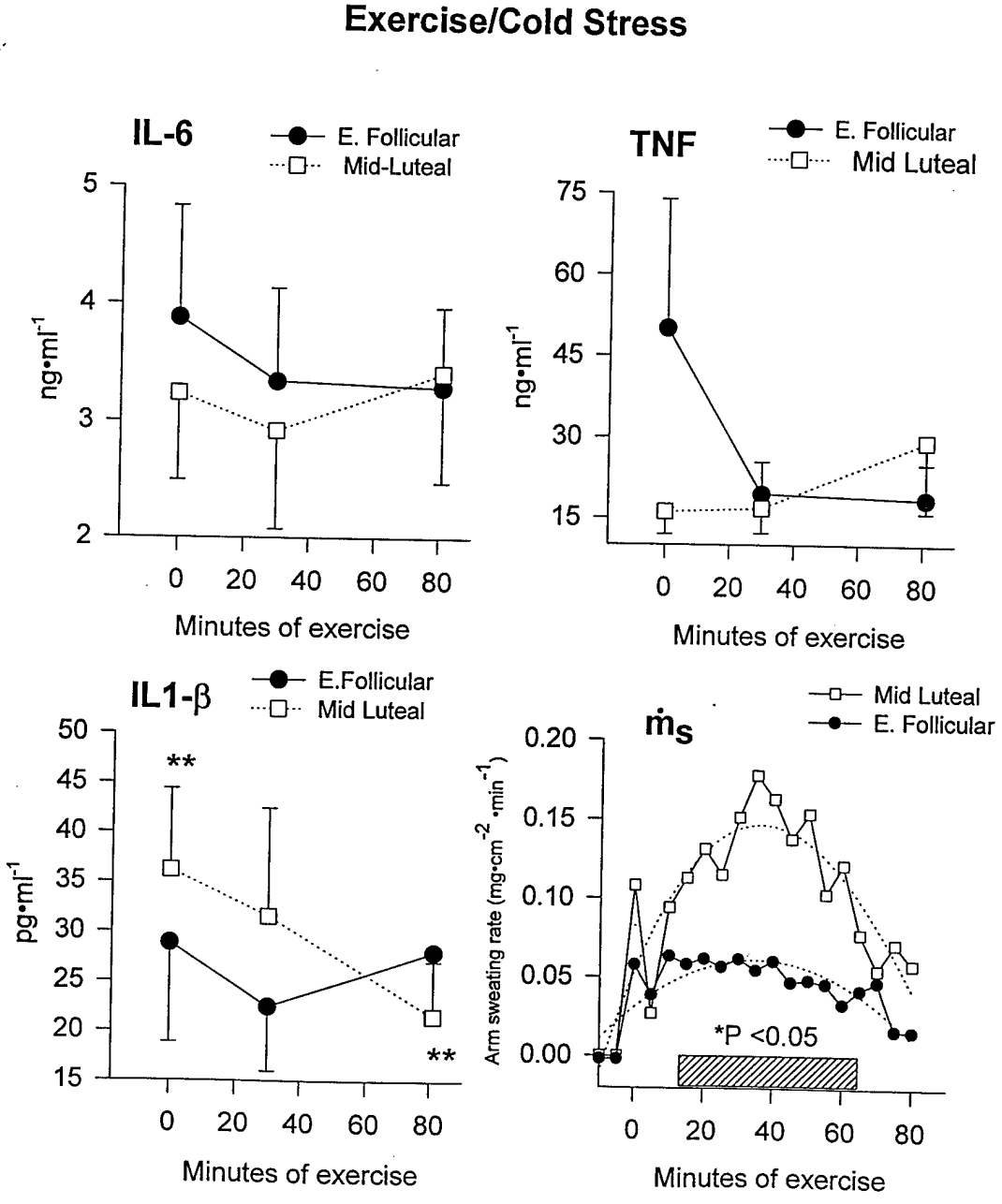


Fig 8. Comparison of four cold stress models to present study algorithm. Right panel shows combined regression analyses without inclusion of Stolwijk-Hardy (S-H) algorithm for shivering.

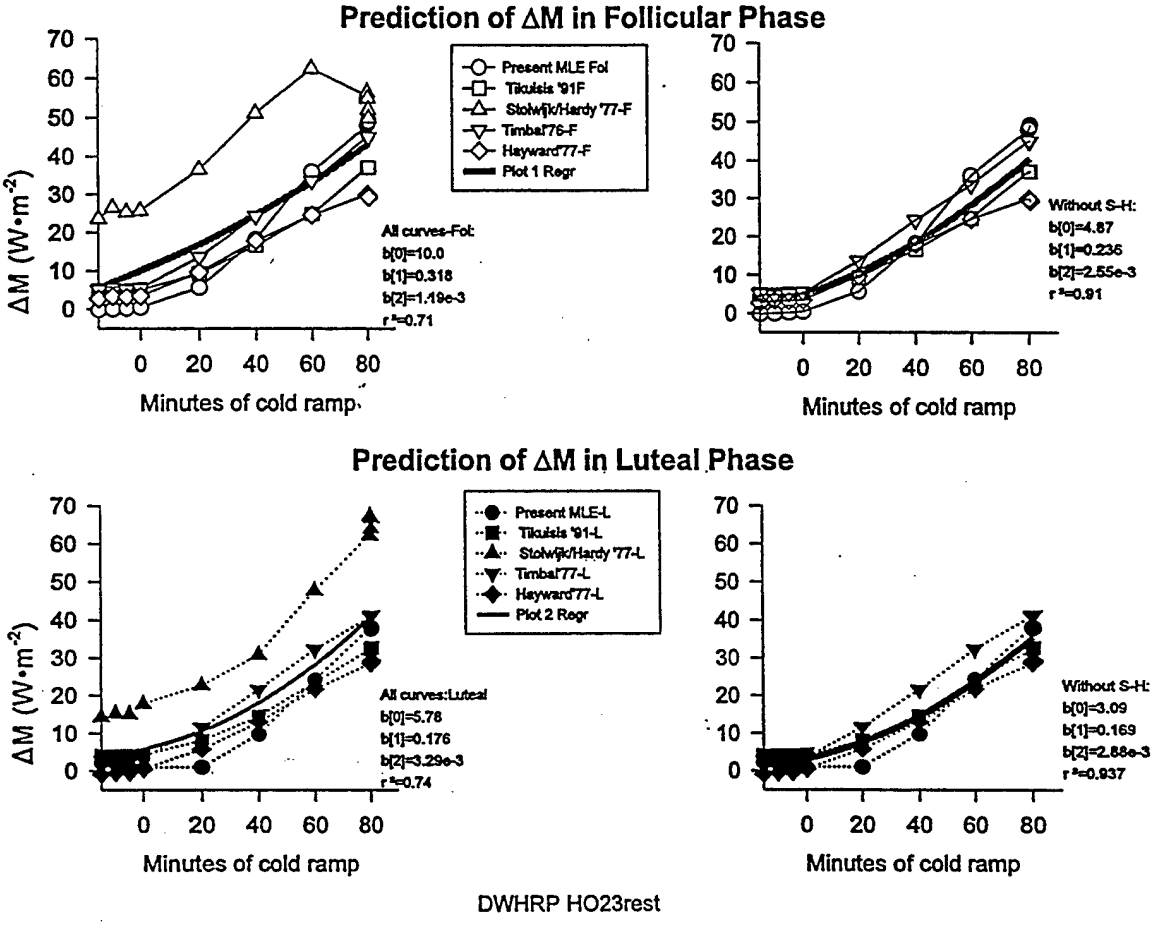
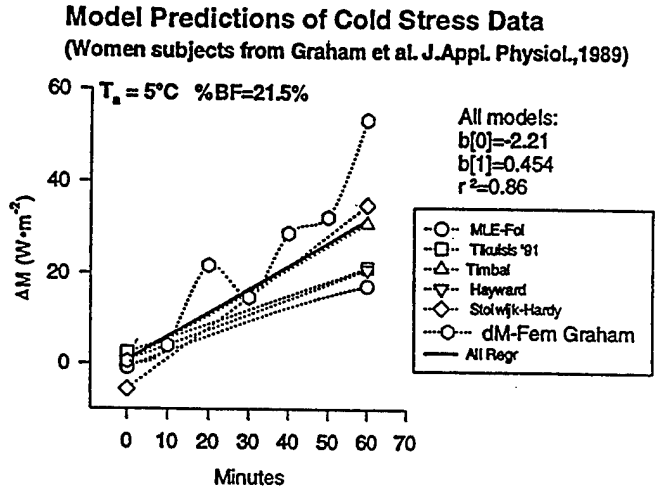
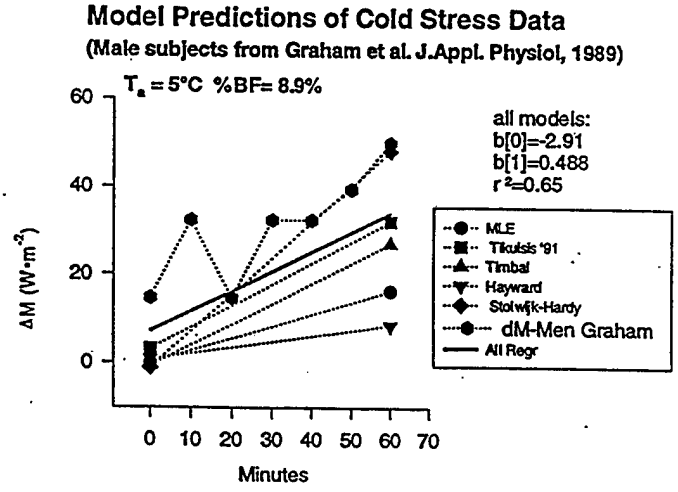


Fig 9. Comparison of model output from the four models and current study algortihm versus experimental data in resting men and women (follicular data only).





PUBLICATIONS FROM GRANT:

1. Shitzer, A., L.A. Stroschein, R.R. Gonzalez, and K.B. Pandolf. Lumped-parameter tissue temperature-blood perfusion model of a cold-stressed finger tip. J. App. Physiol. 80(5):1829-1834, 1996.

2. Shitzer, A. L.A. Stroschein, P. Vital, R.R. Gonzalez, and K.B. Pandolf. Numerical analysis of an extremity in a cold environment including counter-current arterio-venous heat exchange. J. Biomed. Engr. (in press).

3. Shitzer, A., L.A. Stroschein, R.R. Gonzalez, and K.B.Pandolf. Application of a lumped-parameter heat exchange model to cold-induced temperature and blood flow measurements in the finger-tip. J. Thermal Biol. 21(4): 213-222, 1996.

4. Shitzer, A., T.L. Endrusick, L.A. Stroschein, R.F. Wallace, and R.R. Gonzalez. Characterization of a three-phase response in cold-stressed fingers. Eur J. Appl Physiol, 78(2): 155-162,1998.

5. Gonzalez, R.R. and L.A. Blanchard. Thermoregulatory responses to cold transients: Effects of menstrual cycle in resting women. J.App. Physiol. 85:543-553, 1998.

6. Gonzalez, R.R., L.A. Blanchard, and W.F. Allison. Cytokine interaction with the menstrual cycle during cold stress and exercise. *Annals of the New York Academy of Sciences.* (in press)

7. Gonzalez, R.R., L. A. Blanchard, W.F. Allison, and J.A. Gonzalez. Thermoregulatory Responses to Cold Transients: Effects of Two Clothing Systems in Resting Women. USARIEM Technical Report, December, 1996.

8. Kraning, K.K. and R.R. Gonzalez. Scenario: A military/industrial heat strain model modified to account for effects of aerobic fitness and progressive dehydration. USARIEM Technical

Note, March, 1997.

9. Shitzer, A., T.L. Endrusick, L.A. Stroschein, R.F. Wallace, and R.R. Gonzalez. Characterization of a three-phase response in cold-stressed fingers. USARIEM Technical Report, May, 1997.

MEETING ABSTRACTS:

Gonzalez, R.R., L.A. Blanchard, N. Charkoudian, W.F. Allison, and M.A. Kolka. Thermoregulatory Responses to cold transients. Experimental Biology 96, New Orleans, LA, April, 1996. FASEB J, 10:(3): A116, 1996.

Kraning, K. K., Montain, S. J., A computer simulation to predict the physiological effects of progressive dehydration. Experimental Biology 96, New Orleans, LA, April, 1996. FASEB J , 10:A117, 1996.

Allison, W.F., L.A. Blanchard, R.R. Gonzalez, K. Rudolph, and M.J. Kluger. Are Cytokines Mediating an Elevated Core Temperature in the Luteal Phase of the Female Menstrual Cycle? Experimental Biology 97, Washington, D.C. April, 1997.

Gonzalez, R.R., and L. A. Blanchard. Cold Stress in Women-Comparison of Model and Experimental Results with Protective Clothing. Experimental Biology 98, San Francisco, CA, April 18-22, 1998.

Gonzalez, R.R. Thermoregulatory Models and Human Responses to the Environment. Society for Engineering Science-Invited Symposium, SFS98, Washington State Univ-Pullman, WA, September 27-30, 1998.

CONTRACTUAL PERSONNEL:

Dr Avraham Shitzer (Technion University, Haifa, Israel, Senior National Research Council Fellow);

Lovelace Foundation, Albuquerque, NM: Dr Matthew J. Kluger; Dr Karin Rudolph, cytokine analyses;

Dr Joseph Cannon: Penn State University, cytokine analyses;

Dr. Kenneth K. Kraning, USARIEM (deceased).

Mrs. Janet E. Staab, Ms Michelle Mayo, and Ms Jane P. Deluca (USARIEM) general biochemical analyses;

Ms. Nisha Charkoudian (Springfield College student contract); Ms Marissa Volpe (Framingham State University student contract); Ms Mona Sharif (Boston Universtiy student contract); Ms Amy Dunham (Boston University student contract).